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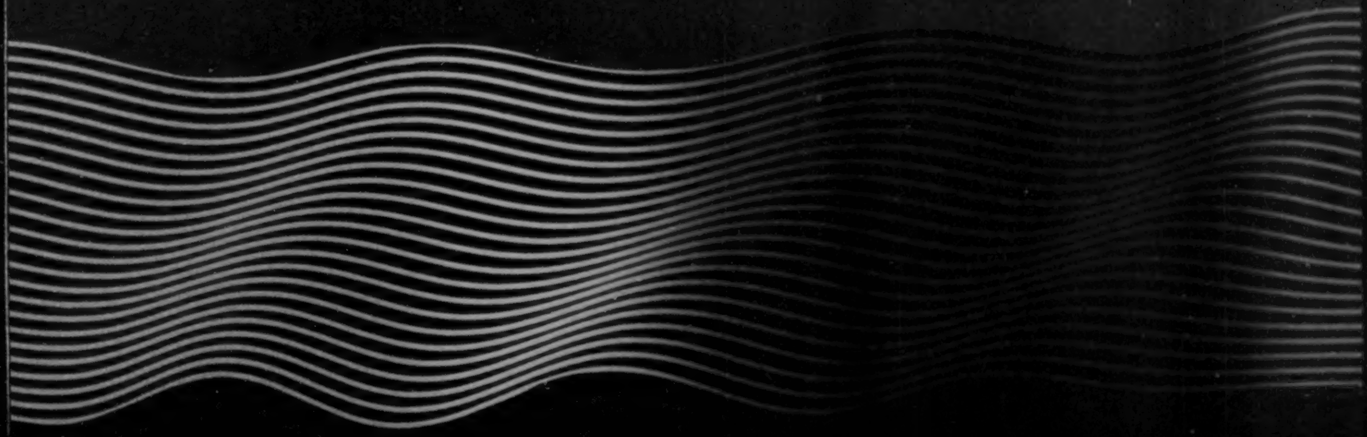

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Marine ecosystem assessment in a fisheries management context

Jason S. Link, Jon K.T. Brodziak, Steve F. Edwards, William J. Overholtz, David Mountain, Jack W. Jossi, Tim D. Smith, and Michael J. Fogarty

Abstract: We examined a suite of abiotic, biotic, and human metrics for the northeast U.S. continental shelf ecosystem at the aggregate, community, and system level (>30 different metrics) over three decades. Our primary goals were to describe ecosystem status, to improve understanding of the relationships between key ecosystem processes, and to evaluate potential reference points for ecosystem-based fisheries management (EBFM). To this end, empirical indicators of ecosystem status were examined and standard multivariate statistical methods were applied to describe changes in the system. We found that (*i*) a suite of metrics is required to accurately characterize ecosystem status and, conversely, that focusing on a few metrics may be misleading; (*ii*) assessment of ecosystem status is feasible for marine ecosystems; (*iii*) multivariate points of reference can be determined for EBFM; and (*iv*) the concept of reference directions could provide an ecosystem level analog to single-species reference points.

Résumé : Nous avons étudié une série de >30 métriques abiotiques, biotiques et humaines de l'écosystème de la plate-forme continentale du nord-est des É.-U. à l'échelle de l'association, de la communauté et du système couvrant plus d'une trentaine d'années. Nos objectifs principaux étaient de décrire l'état de l'écosystème, d'améliorer la compréhension des relations entre les processus principaux de l'écosystème et d'évaluer des valeurs de référence potentielles à utiliser dans une approche écosystémique de gestion de la pêche (EBFM, « ecosystem-based fisheries management »). Pour ce faire, nous avons examiné les indicateurs empiriques de l'état de l'écosystème et nous avons utilisé les méthodes multidimensionnelles courantes pour décrire les changements dans le système. Nous avons trouvé que (*i*) il faut une série de métriques pour caractériser de façon précise l'état de l'écosystème et, inversement, la concentration sur un petit nombre de métriques peut induire en erreur; (*ii*) il est possible d'évaluer l'état des écosystèmes marins; (*iii*) on peut déterminer des valeurs de références multidimensionnelles pour usage dans l'EBFM; et (*iv*) le concept de direction de repérage peut fournir à l'échelle de l'écosystème un analogue aux valeurs de référence utilisées dans le cas d'une seule espèce.

[Traduit par la Rédaction]

Introduction

There is considerable interest in ecosystem-based fisheries management, as evinced by several recent reports, articles, and books (discussed in Link 2002). The current relevance and awareness of an ecosystem approach has been attributed to conflicting stakeholders or legislation (i.e., allocation of biomass issues), debate over the most important processes in an ecosystem, limitations of single-species management, or simply the use of an ecosystem perspective to justify a wide variety of positions.

Considering factors that impact marine resource populations in a context beyond just the species level has a long and noted history in fisheries science. Multispecies, trophodynamic, environmental, and ecosystem considerations are certainly not novel (e.g., Baird 1873; Lankester 1884). Several approaches were developed in the 1970s and 1980s to extend single-species models by incorporating some of these broader considerations in a fisheries management context (e.g., May et al. 1979; Mercer 1982; Daan and Sissenwine 1991). Yet despite the attention given to this problem during the past century and a half, many basic issues remain unaddressed. The implementation of broader ecosystem considerations has not yet become widespread in fisheries management (particularly in large, marine ecosystems), although it has been recently advocated and even mandated.

Several metrics exist that can indicate ecosystem status independent of specific objectives (e.g., Rice 2000; Brodziak and Link 2002). These "emergent" metrics may be at the multispecies (= community), food web (= important trophic links), aggregate (= groupings of related taxa), or whole system (= entire open ecosystem) level. We view any attempt to ascertain and understand the status of an ecosystem as sequential, involving multiple metrics and the interdisci-

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Table 1. Metrics examined in this study and the extent of these time series.

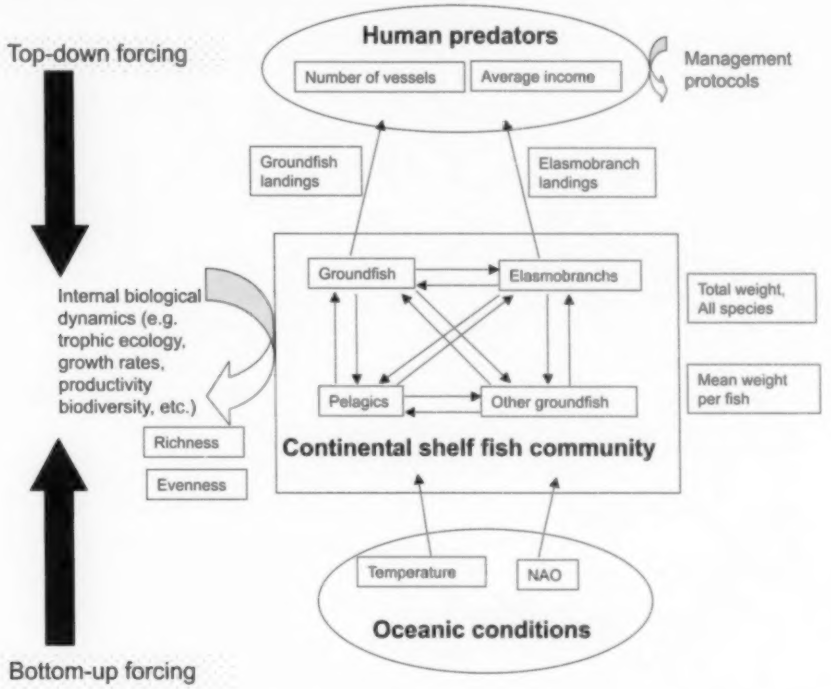
Metric	Start year	End year	Time range	Source	In models	Indexes: process
Biotic metrics						
Resource dynamics						
Total biomass index	1963	2000	37	BTS	N (Fig. 2*a*)	System production
Relative abundance of NE species groups	1963	1999	36	BTS	N	Aggregate production, biomass allocation
Principal groundfish abundance	1963	1999	36	BTS	Y (Fig. 2*c*)	Aggregate production
Elasmobranch abundance	1968	1999	31	BTS	Y (Fig. 2*b*)	Aggregate production
Other groundfish abundance	1963	1999	36	BTS	Y (Fig. 2*c*)	Aggregate production
Principal pelagics abundance	1968	1994	31	BTS	Y (Fig. 2*c*)	Aggregate production
Georges Bank cephalopod abundance	1963	1999	36	BTS	N	Aggregate production
Gulf of Maine total species richness	1963	2000	37	BTS	N (Fig. 3*b*)	Diversity, biomass allocation
Gulf of Maine species evenness	1963	2000	37	BTS	N	Diversity, biomass allocation
Georges Bank total species richness	1963	2000	37	BTS	Y (Fig. 3*b*)	Diversity, biomass allocation
Georges Bank species evenness	1963	2000	37	BTS	Y	Diversity, biomass allocation
Mid-Atlantic Bight total species richness	1963	2000	37	BTS	N (Fig. 3*b*)	Diversity, biomass allocation
Mid-Atlantic Bight species evenness	1963	2000	37	BTS	N	Diversity, biomass allocation
Internal dynamics						
Mean animal length on Georges Bank	1963	2000	37	BTS	N (Fig 2*d*)	Allometric dynamics
Mean animal weight, shelf-wide	1963	2000	37	BTS	Y	Allometric dynamics
Silver Hake linkage density	1973	1999	26	FH	N (Fig. 3*a*)	Trophic dynamics, energy flow
Percentage cannibalism of Gadids	1973	1998	25	FH	N	Trophic dynamics
Percent of fish mature at age-1 and age-2 for Georges Bank haddock and cod	1963	1997	34	BTS	N	Population dynamics, allometric dynamics
Consumption of pelagic species by major predators	1977	1997	20	FH, BTS	N	Biomass allocation, energy flow, trophic dynamics
Abiotic metrics						
Shelf-wide surface and bottom water temperature anomalies	1963	2000	37	BTS	N	Forcing physics
5-year average of NAO index	1823	2000	177	OCE	N	Long-term forcing
NAO index	1960	2000	41	OCE	Y (Fig. 3*c*)	Long-term forcing
Bottom temperature anomalies in the Gulf of Maine	1963	2000	37	BTS	Y (Fig. 3*d*)	Forcing physics
Bottom temperature anomalies on Georges Bank	1963	2000	37	BTS	Y	Forcing physics
Bottom temperature anomalies in the northern Mid-Atlantic Bight	1963	2000	37	BTS	Y	Forcing physics
Bottom temperature anomalies in the southern Mid-Atlantic Bight	1963	2000	37	BTS	Y (Fig. 3*d*)	Forcing physics
Human metrics						
Otter trawl landings by species	1964	2000	36	OBS	Y (Fig. 4*a*)	Humans as predators
Otter trawl revenue by species	1964	2000	36	OBS	Y (Fig. 4*b*)	Humans as predators
Number of otter trawl vessels by size class	1964	2000	36	OBS	Y (Fig. 4*c*)	Humans as predators
Otter trawl income in year 2000 value	1964	2000	36	OBS	N	Humans as predators
Average otter trawl income in year 2000 value	1964	2000	36	OBS	Y (Fig. 4*d*)	Humans as predators

Note: For the source, BTS = bottom trawl survey, FH = food habits, OBS = observer and port agent commercial landings database, OCE = oceanographic database, REG = regulations implemented. Whether or not the metric was used in the multivariate statistical models is denoted (Y/N), and if appropriate, the corresponding figure is noted. The general process indexed by each metric is also listed.

plinary integration, synthesis, and interpretation of these ecosystem metrics. The metrics need to be sensitive to change, directional, general enough to be useful, feasible to measure, and able to incorporate uncertainty. It takes multiple time series of metrics and associated monitoring to assess the status of a system to interpret these metrics in any sort of meaningful management context.

Our goals were to examine some common ecosystem metrics to assess the status of the U.S. northwest Atlantic continental shelf ecosystem and to determine whether any of these metrics, or a combination thereof, could serve as useful ecosystem reference points analogous to single-species reference points and control rules common in fisheries management. We present example metrics representative of the major processes in this ecosystem. Additionally, we present multivariate analyses of ecosystem status and evaluate their

Fig. 1. Conceptual model of major ecosystem processes. All metrics in the solid boxes were used in the principal components and canonical correlation analyses.


Top-down forcing
Human predators
Number of vessels
Average income
Management protocols
Groundfish landings
Elasmobranch landings
Internal biological dynamics (e.g. trophic ecology, growth rates, productivity biodiversity, etc.)
Groundfish
Elasmobranchs
Pelagics
Other groundfish
Total weight, All species
Mean weight per fish
Richness
Evenness
Continental shelf fish community
Temperature
NAO
Oceanic conditions
Bottom-up forcing

utility as ecosystem-level reference points. We view this as an initial step in the iterative process of developing an approach for ecosystem-based fisheries management.

Methods

Data sources—empirical indicators

We used existing databases and analyses to estimate a suite of metrics, including human, abiotic, and biotic factors (Table 1). The majority of these databases are associated with the Northeast Fisheries Science Center's (NEFSC's) long-standing bottom trawl surveys. As such we will preclude a detailed discussion of the methodology involved with collecting these data and estimation of the various parameters. For more details on most biotic metrics, see Azarovitz (1981) and NEFSC (1999). For the community diversity metrics, we used what was captured by the survey trawl gear. We grouped the groundfish into major groundfish (haddock (*Melanogrammus aegelfinus*), cod (*Gadus morhua*), and yellowtail flounder (*Limanda ferruginea*)) and others. For more details about trophic metrics, see Link and Almeida (2000) and Overholtz et al. (2000). For more details about physical oceanographic metrics, see Mountain (1989), Taylor and Bascuñán (2001), and Brodziak and Link (2002). For landings, commerical fisheries information, and related human parameters, see NEFSC (1999), Brodziak and Link (2002), and S.F. Edwards (NMFS, Woods Hole, MA 02543, U.S.A., unpublished data).

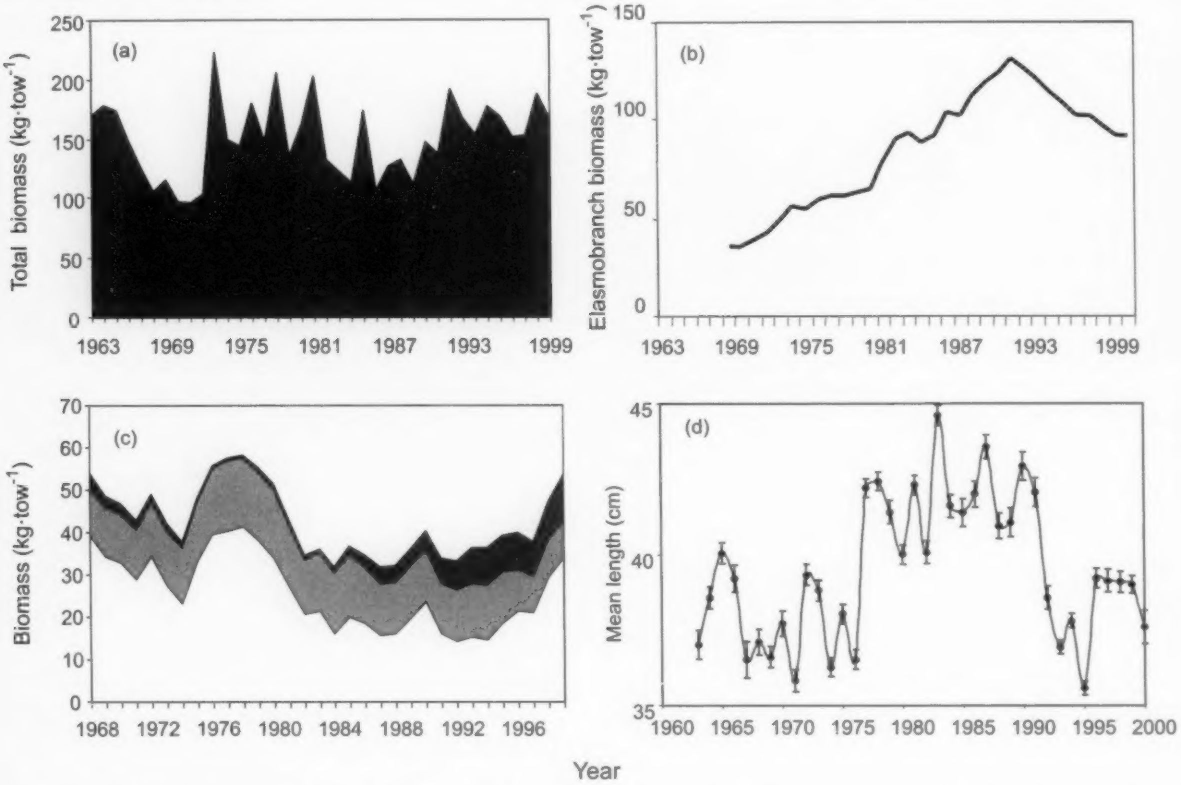
We selected various human, abiotic, and biotic metrics from a suite of many possible (Table 1) that correspond to the major processes in the northwest Atlantic ecosystem (Fig. 1). We recognize that the other metrics not selected may similarly quantify major processes within this ecosystem. The selected metrics are not meant to be exhaustive, but rather representative of the major components of this ecosystem. The metrics are also useful as proxies for various activities and processes within the ecosystem, for example, the number of vessels can serve as an indicator of the overall fishing effort potentially occurring in the system. For a broader selection and discussion of metrics for this ecosystem, see Link and Brodziak (2002).

We calculated quintiles of the empirical distribution for the representative metrics. By color coding various quintiles, we obtain a perspective of recent years relative to the overall average for each metric (Halliday et al. 2001; Brodziak and Link 2002; Caddy 2002). For example, if a metric exhibits values in the highest quintile during the most recent year or set of years, we would code that year as black, indicative that this metric value was one of the highest (i.e., positive) values observed. By examining the full suite of metrics, one can visualize the status of an ecosystem relative to historical levels. We assigned the highest quintiles black, the second dark gray, the third cross-hatched, the fourth vertically lined, and the lowest white. This approach differs from the typical "traffic light" approaches (Halliday et al. 2001; Caddy 2002) in that we did not assign a value judgement (i.e., good or bad) to any particular quintile.

Data analysis—statistical models

We used principal components analysis (PCA; Legendre and Legendre (1998) or Jongman et al. (1999); on the corre-

Fig. 2. (*a*) Total fish biomass of the entire northeast shelf ecosystem as estimated from the Northeast Fisheries Science Center bottom trawl surveys. These estimates include all finfish species. (*b*) Major trends in aggregate biomass for the elasmobranchs. (*c*) Major trends in biomass for principal groundfish (open), other groundfish (shaded), and principal pelagics (solid). (*d*) Mean length (± standard error) of all fish species on Georges Bank.



lation matrix) on the selected metrics to reduce the number of multivariate dimensions to a smaller set of linear combinations that explained the most variance, provided some delineation of key processes (e.g., biotic tradeoffs, abiotic forcing functions, human intervention, etc.) effecting the ecosystem, and tracked major trends across the time series. The conceptual model that we used for the PCA included abiotic and human factors that can influence the biotic metrics as well as other biotic metrics that index internal regulatory biological processes (Fig. 1).

In addition to the simple descriptive PCA model, we used canonical correlation analysis (CanCorr; Jongman et al. (1999) or Legendre and Legendre (1998)) to examine the linear relationships between multivariate response (biotic) and explanatory (human and abiotic) metrics. Although the general conceptual model that we used was the same as in the PCA analysis, the CanCorr provided an evaluation of possible dependance among metrics. CanCorr is a multivariate equivalent to multiple linear regression that used the biotic metrics as response variables.

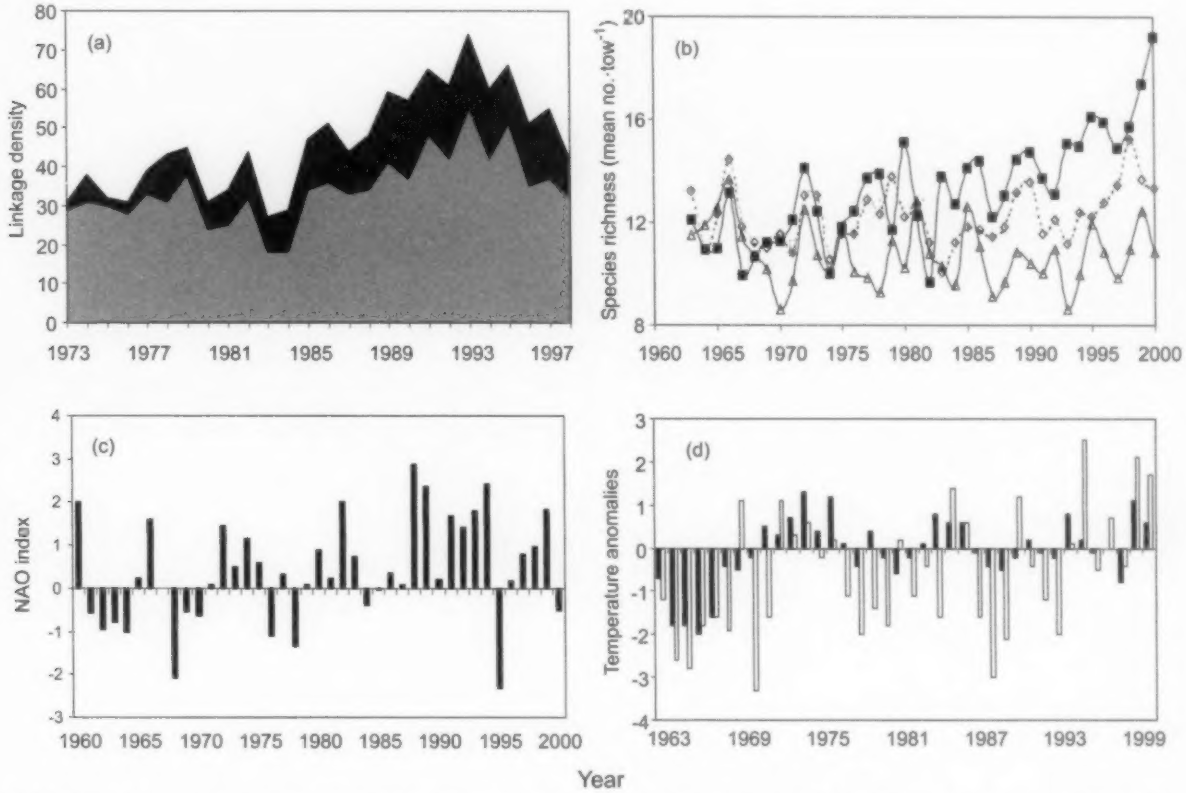
The PCA and CanCorr results were further explored to determine if the first two principal component weights or the first two canonical axes could be used to describe ecosystem status as a multivariate analogue to single-species reference points. From the PCA, we grouped annual scores plotted in phase–space into related quadrats to represent major "regimes" or "stanzas" of this ecosystem. We then examined the multivariate trajectory and potential dependent relationships among the ecosystem metrics using the CanCorr results. By examining the results from both methods, we could then ascertain ecosystem status relative to some desirable state in multivariate space. For example, if there was a strong relationship between the first canonical axes from the CanCorr analysis, we could use that relationship to suggest possible manipulations in the explanatory axis (and composite metrics) that would result in changes to the response axis corresponding to a desirable locale in the phase–space plot from the PCA.

Results

Empirical indicators

The average total fish biomass of the ecosystem (Fig. 2*a*), after the initial years of the survey, has effectively shown no trend, within a range of variation, across the time series. However, allocation of fish biomass across different groups has varied (Figs. 2*b*, 2*c*). In more recent years, the planktivore guild has become much more dominant (i.e., increases in juvenile dogfish and small pelagics such as herring and mackerel). This observation simply suggests that within the

Fig. 3. (*a*) Linkage density (number of predators, solid; number of prey, shaded) for silver hake (*Merluccius bilinearis*). (*b*) A measure of diversity (species richness) for the Gulf of Maine (solid squares), Georges Bank (open diamonds), and the Mid-Atlantic Bight (open triangles). (*c*) The North Atlantic Oscillation (NAO) index (adapted from Brodziak and Link 2002). (*d*) Temperature anomalies for two regions of the ecosystem, the Gulf of Maine (solid) and the southern Mid-Atlantic Bight (open) (adapted from Mountain 1989; Taylor and Bascuñán 2001).



limitations of thermodynamics, differential effects of fishing or the environment alter the internal dynamics (e.g., trophic dynamics, community structure, productivity, etc.) of the ecosystem, ultimately regulating the allocation of biomass within the ecosystem.

Most metrics exhibit a change across the time series. Mean length (Fig. 2*d*), number of silver hake species interactions (Fig. 3*a*), Gulf of Maine and Mid-Atlantic Bight species richness (Fig. 3*b*), the North Atlantic Oscillation (NAO; Fig. 3*c*), temperature (Fig. 3*d*), and most species landings and associated fisheries income (Fig. 4) have generally changed to values very different in the 1990s vs. the 1980s, suggestive of broad-scale changes in this ecosystem. The consistent peak in many metrics during the late 1970s to early 1980s (Figs. 2–4) is also interesting; this peak occurred for 17 out of the 35 metrics that we examined, and conversely a depressed series of values occurred for 10 others during the same time. This peak may represent some form of cyclicity in the biotic components of the ecosystem, similar to the abiotic features (e.g., temperature, NAO; Figs. 3*c*, 3*d*), or a shift to a new steady state. No one metric best described the status of the ecosystem, even though many of the metrics demonstrated similar trends and many of the metrics similarly captured the directionality of key processes and relationships. Examining just one or a few (e.g., pelagic biomass, elasmobranch biomass, silver hake linkage density, species richness in just one region) may be misleading. Given the consistent change in multiple metrics from this time period, there was likely a large event or series of events perturbing the system during the late 1970s to early 1980s.

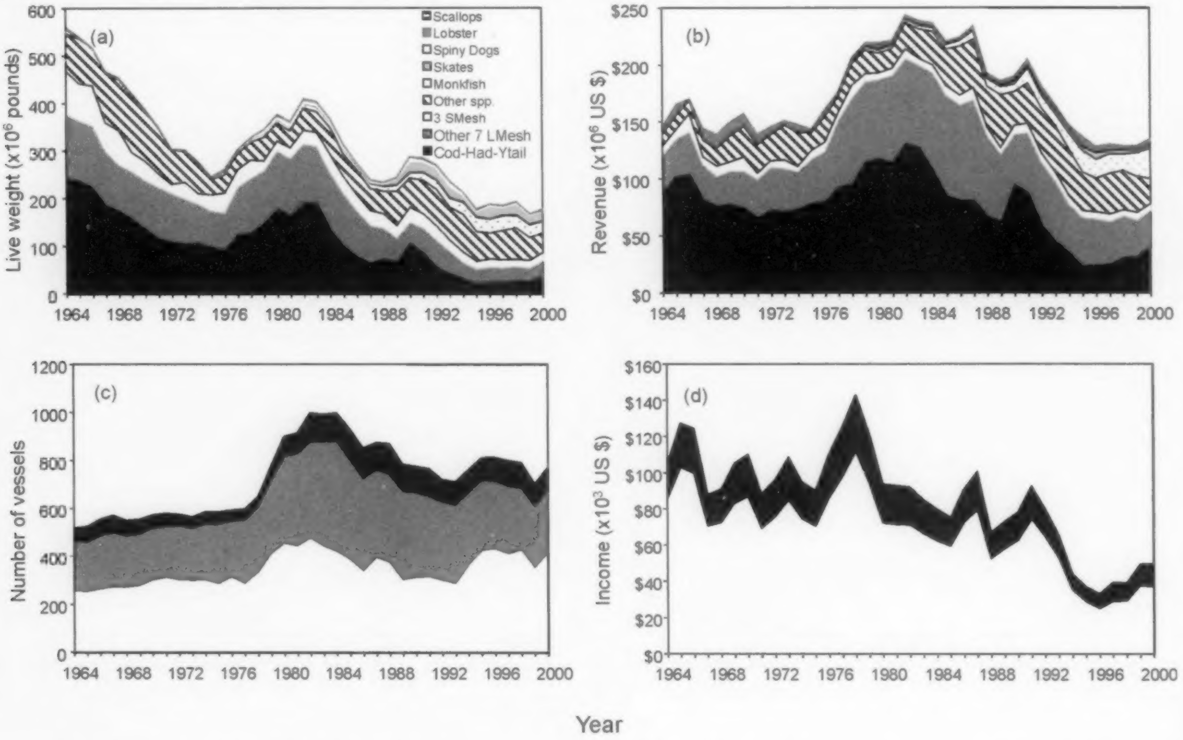
The quintile approach demonstrates that conditions have changed across the time series (Table 2). Metrics such as pelagic species biomass, the NAO, southern Mid-Atlantic Bight temperatures, and elasmobranch landings are more positive in recent years than most of the other metrics. The previously described peaks during the early 1980s are also apparent. This approach does not present any new information, but packages the information into one simple, intuitive graphic that can be readily digested by multiple stakeholders (Schiller et al. 2001; Halliday et al. 2001; Caddy 2002).

Obviously, many of these metrics are correlated. There are numerous similarities (or inversions) in the directionality and relative magnitude in the major trends observed for most metrics. However, the strength and interdependence of the relationships among the metrics are unknown.

Statistical models

The first two axes of the PCA explained 48% of the total

Fig. 4. (*a*) New England otter trawl landings by species or species groups. (*b*) Total otter trawl revenue (adjusted for inflation to year 2000 US$) by species. See the shading legend in Fig. 4*a*. (*c*) Total number of vessels fishing in the otter trawl fleet. Tonnage class 2 (TC2) is open, TC3 is shaded, and TC4 is solid. (*d*) Average otter trawl income (adjusted for inflation to year 2000 US$) per vessel (open) and crew (solid) (all adapted from S.F. Edwards, unpublished data).



variance (Fig. 5), and the first four principal components (PC) explained 70%. Interestingly, no one factor dominated the weightings. The first PC can be thought of as an index of low groundfish biomass and landings, low profits, low species evenness, and small fish at negative scores. Conversely, positive first axis scores corresponded to high skate landings and high elasmobranch and pelagic biomass. The first PC axis scores have generally increased across time, corresponding to increases in elasmobranch biomass–landings, pelagic biomass and declines in groundfish landings, fish size, and income (Figs. 2–4). The second PC can be thought of as an index of temperature and groundfish biomass (positive scores) or conversely total fishing effort (i.e., total number of vessels, landings). The second PC axis scores were lower during the mid-1980s, corresponding to the peak in the number of vessels, groundfish and dogfish landings, cooler temperatures, and declining groundfish biomass (Figs. 2–4).

When the PC scores for the first two axes are plotted throughout time, an interesting trajectory develops (Fig. 6). The pattern generally tracks from the upper left quadrat (I, negative PC1, positive PC2) counter clockwise to the upper right quadrat (IV, positive PC1 and PC2). We categorically group the annual scores into three regions corresponding to conditions noted in Table 2. It is uncertain if the system could even return from the current conditions in the upper right quadrat to the conditions in the upper left.

The first canonical axis of the explanatory variables is primarily defined similar to the second PC axis described above, with positive scores corresponding to high elasmobranch landings, number of vessels, and Mid-Atlantic Bight temperature (Fig. 7*a*). Conversely, negative scores correspond to high groundfish landings. The second explanatory canonical axis effectively is an index of income, the number of vessels, and other groundfish vs. most other species landings. Interestingly, this second axis had neutral scores for various measures of temperature.

The first canonical axis of the response metrics simply contrasts groundfish vs. pelagic biomass (Fig. 7*b*). The second axis contrasts evenness vs. individual fish weight and other groundfish. The univariate statistics show that scores for other groundfish biomass ($R^2 = 54.3\%$, $P < 0.0817$), groundfish biomass ($R^2 = 67.5\%$, $P < 0.0072$), pelagic biomass ($R^2 = 72.6\%$, $P < 0.0019$), and species evenness ($R^2 = 69.0\%$, $P < 0.0050$) are significantly different than 0 for both axes.

The first two axes of the CanCorr analysis explain 81% of the total variance. The first two eigenvalues ($p < 0.003$) and summary statistics are statistically significant (Wilk's $\lambda = 0.00118$, df = 88, 88.149, $p < 0.032$). The relationship between the first canonical axes has an R^2 of 93.9% (Fig. 7*c*). Coupled with the PCA trajectory (Fig. 6), this relationship can roughly be interpreted to mean that after a period of high groundfish landings, then low groundfish landings, high elasmobranch landings, high numbers of vessels, and high

Table 2. Summary of 5-year averages of abiotic, biotic, and human metrics to ascertain qualitative status of the northeast shelf ecosystem characteristics. The patterns (see Color code of metric) correspond to the different quintiles that these metrics exhibit with respect to the historical time series. The quadrat and region refer to the location in multivariate space of the PCA (principal component analysis) time trajectory for each period of time (Fig. 6). MAB, Mid-Atlantic Bight; N/A = data not available for that year.

Quadrat		IV	IV	III	II	II	I	I
Multivariate region		C	C	B	B	A	A	A
Metric	Value in 2000	Average 1995-1999	Average 1990-1994	Average 1985-1989	Average 1980-1984	Average 1975-1979	Average 1970-1974	Average 1965-1969
Abiotic metrics								
North Atlantic oscillation	□	⊠	■	▒	▒	▥	⊠	▥
Gulf of Maine bottom temperature	⊠	▒	⊠	▒	⊠	⊠	▒	⊠
Georges Bank bottom temperature	⊠	▒	▒	⊠	⊠	▒	▒	□
N MAB bottom temperature	N/A	▒	■	⊠	▥	⊠	⊠	□
S MAB bottom temperature	N/A	■	⊠	⊠	⊠	⊠	⊠	▥
Biotic metrics								
Total biomass	N/A	▒	▒	⊠	⊠	▒	⊠	⊠
Mean weight per fish	N/A	⊠	⊠	⊠	⊠	⊠	▒	■
Groundfish	N/A	⊠	□	□	⊠	▒	⊠	■
Other groundfish	N/A	□	⊠	▒	■	■	⊠	□
Elasmobranchs	N/A	▒	■	■	■	■	□	□
Pelagics	N/A	■	▒	⊠	⊠	□	⊠	⊠
Georges Bank species richness	■	■	⊠	⊠	⊠	▒	⊠	⊠
Georges Bank species evenness	⊠	⊠	⊠	⊠	⊠	▒	■	■
Human metrics								
Domestic groundfish landings	□	□	⊠	⊠	■	⊠	⊠	■
Domestic elasmobranch landings	■	■	■	▒	▒	■	■	□
Average otter trawl income	□	□	⊠	⊠	⊠	■	▒	■
Number of otter trawl vessels	⊠	▒	⊠	▒	■	▥	▥	□

	Percentiles of empirical distribution				
	100-80th	80-60th	60-40th	40-20th	20-0th
Color code of metric	■	▒	⊠	▥	□

Mid-Atlantic Bight temperatures correspond to conditions of low groundfish biomass and high pelagic biomass. The relationship between the second canonical axes has an R^2 of 70.8% (Fig. 7*d*). This relationship can roughly be interpreted to mean that when there is high effort (number of vessels) that switch from groundfish to other species of groundfish and still maintain a profit, then the biomass of other groundfish and the size of all fish begin to decline, altering species evenness for the ecosystem. The fishing fleet is effectively targeting whatever species are abundant in the system to the point that, coupled with environmental change, the targeted species begin to decline. Said another way, these results are a multivariate expression of sequential depletion.

Discussion

Empirical indicators

The status of an ecosystem can be assessed. Assessing ecosystems is not novel for terrestrial, freshwater, or wetlands ecosystems (e.g., Suter 1993; Hall 2001; Schiller et al. 2001). Additionally, it is not novel to assess the status of single-species fish stocks (e.g., Hilborn and Walters 1992; Quinn and Deriso 1999). Yet only a few recent examples have recognized the feasibility of actually assessing major components of marine ecosystems (Boyd and Murray 2001; Eisenack and Kropp 2001; Pitcher and Preikshot 2001). From this assessment of the northwest Atlantic ecosystem, we have an improved picture of the magnitude and relationships within the system. We recognize that there are other components of this ecosystem not included in our analyses (for logistical reasons; e.g., primary or secondary production, whale biomass, etc.), and it would be important to include them in further analyses. Yet the assessment we provide is not trivial given the spatiotemporal scale of our study. Although many marine ecosystems have well-documented, generalized patterns (e.g., water currents, seasonal warming, trophic relationships, etc.), these generalizations can amalgamate and obfuscate real-time changes in an

Fig. 5. The first two principal components with loadings from the various metrics in the model. The first two axes explain approximately 50% of the variation.

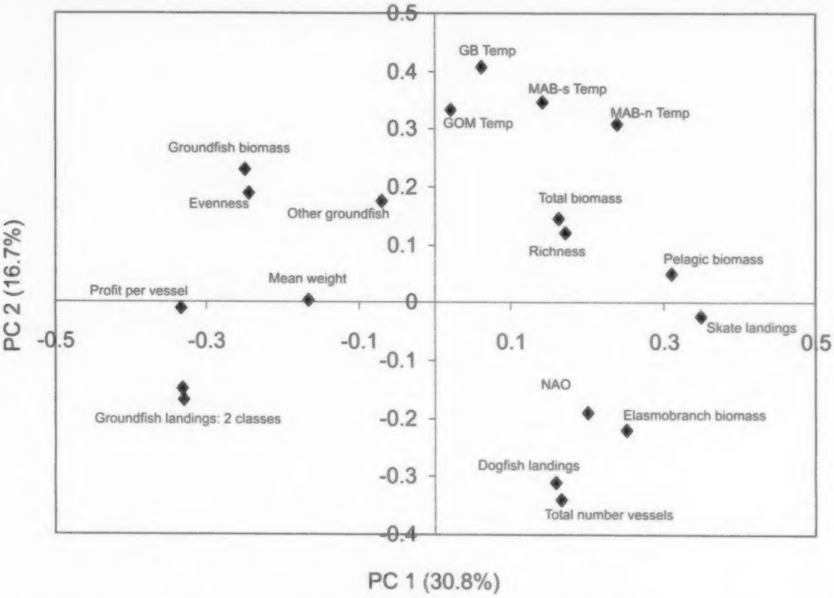
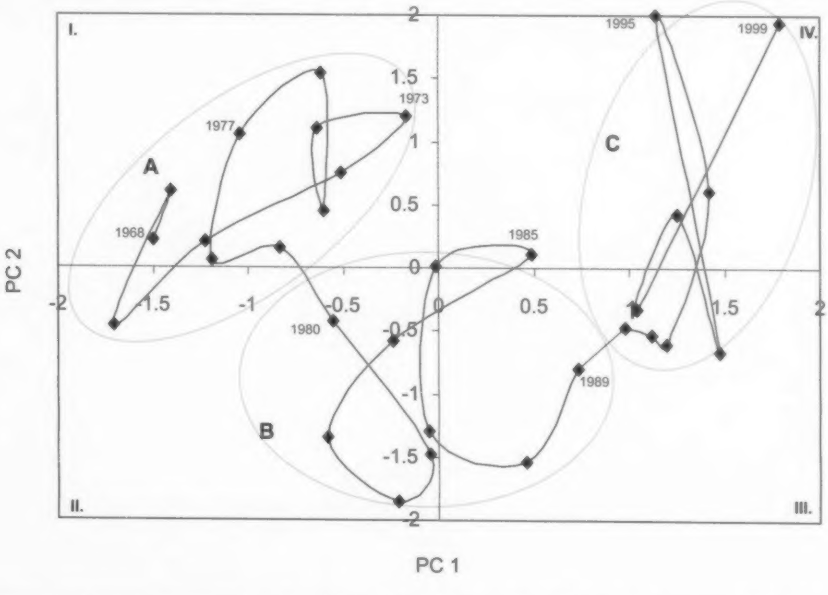


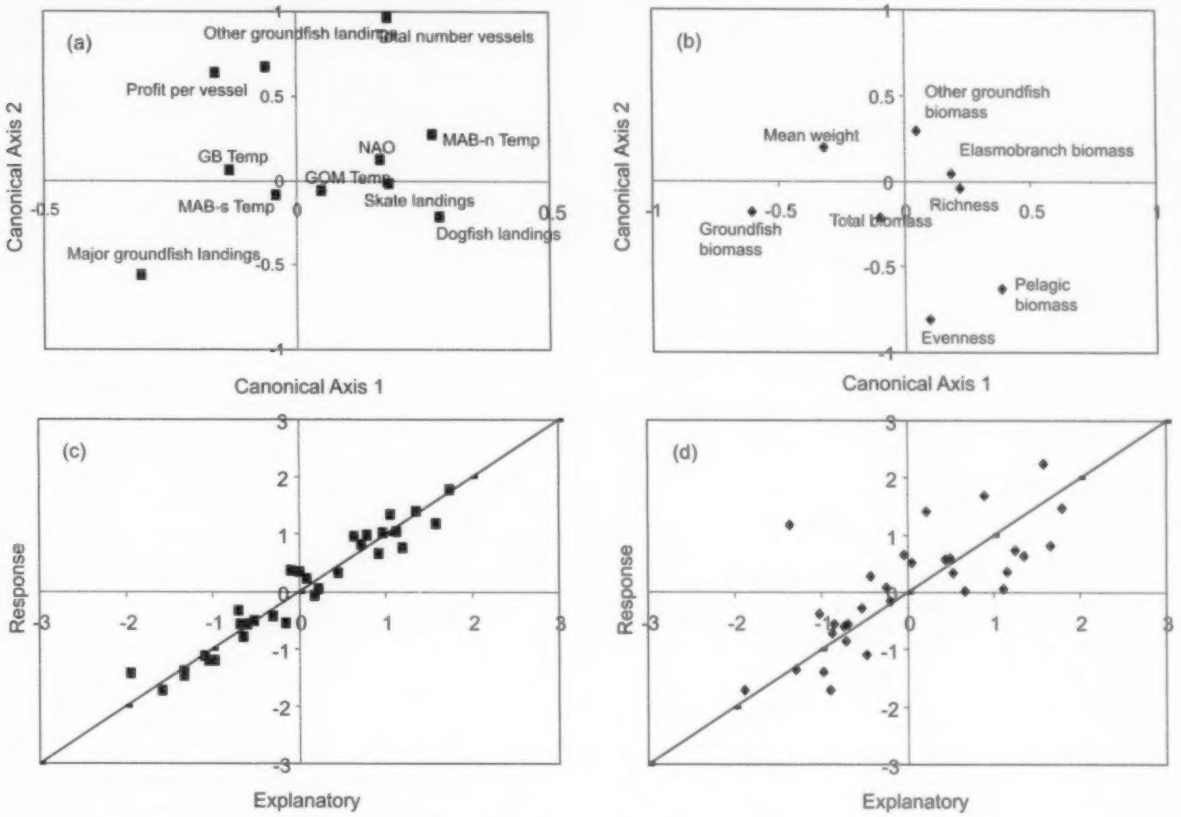
Fig. 6. Scores for each year on the first two principal components, indicating the systemic trajectory. Roman numerals represent quadrat number, and the ellipses represent categorical groupings of similar scores (A, B, and C).



ecosystem. We recommend regularly assessing the status of marine ecosystems much the same as single-species fish stocks are assessed, and we assert that it can be done.

Our results confirm prior studies that focused on specific processes in this ecosystem. For example, we knew that the fish community composition changed drastically (Fogarty and Murawski 1998), that temperature was strongly correlated with the distribution of certain species (Mountain and Murawski 1992), that consumption of key species, energy flows, and related trophic dynamics have altered (Overholtz et al. 2000; Link and Garrison 2002), that diversity changed (Brodziak and Link 2002), and that the size of fish has declined (Murawski and Idoine 1992). The peak observed for many metrics during the late 1970s to early 1980s corresponds to the passage of the first Magnuson–Stevens Fisheries Conservation and Management Act in the late 1970s,

Fig. 7. (*a*) The first two canonical axes for the explanatory metrics, with loadings for said metrics. (*b*) The first two canonical axes for the response metrics, with loadings for said metrics. (*c*) Canonical correlation for between the first axes; R^2 = 93.9%. (*d*) Canonical correlation for between the second axes; R^2 = 70.8%.



which resulted in the expansion of the domestic fleet and a subsequent increase in groundfish landings beyond sustainable levels. Yet this work is distinct from those that focus on a single process in that it integrates all of these considerations at once and attempts to quantitatively determine the importance of numerous processes simultaneously. From that perspective, we view this holistic approach as providing additional (and by all means complementary) information beyond traditional single-species fisheries approaches.

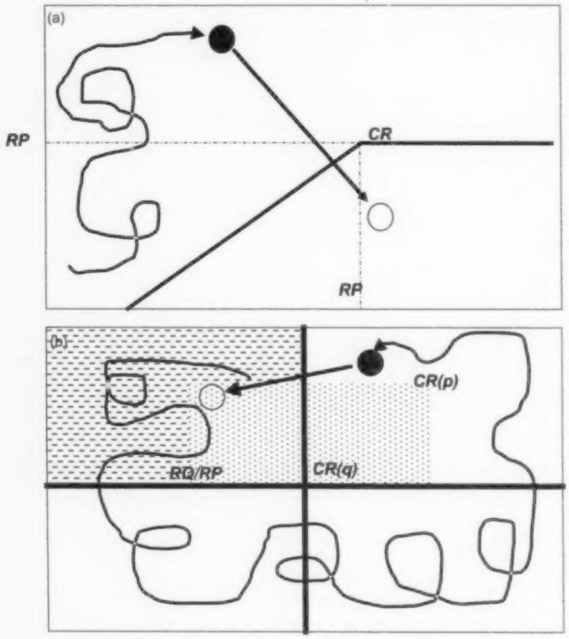
Interpretation of the multivariate relationships identified the relative importance of major processes in this ecosystem. Our results indicate that fishing activities, loosely measured here as landings, profit, or total number of vessels, were the primary factors altering the biotic component of this ecosystem. Yet changes in the environment (e.g., warmer temperatures, long-term climate forcing, etc.) or internal biological processes (i.e., how biomass is allocated among the remaining, noncaptured organisms) can influence the status of the ecosystem. More importantly, now that landings, income, and the number of vessels are declining and management measures have been enacted (e.g., area closures on Georges Bank, mesh size limits, etc.), the abiotic and internal biological processes may become more important factors determining the contemporary status of this ecosystem.

Statistical models

Ecosystem analogs to single-species reference points exist. Other researchers have reached similar conclusions (e.g., Constable 2001; Sainsbury and Sumaila 2001; Schiller et al. 2001). The coefficient weightings in our analyses are consistent with the conceptual model of how this ecosystem functions. One can map current or desired conditions to multivariate phase–space as a way of ascertaining the status of the system relative to some prescribed ecosystem goal. Depending on the value or location of the multivariate score relative to a multivariate ecosystem point of reference, or even reference quadrat, an appropriate action may be then become apparent. The PCA and CanCorr are statistical approaches that can provide reference points and combined may work as ad hoc control rules on an interim basis.

We provide one, admittedly simplistic, approach of how these multivariate analyses could be used in a management context. First, we recommend obtaining values for the multiple metrics for a particular year and (or) situation. Then inserting the values of those metrics into the linear equation combinations derived from the PCA and (or) CanCorr analyses would produce a score for that year. Depending on which reference quadrat (or fraction thereof) the score resides in, appropriate management action could then be taken.

Fig. 8. (*a*) An example of single-species reference points (RP), control rule (CR), and direction (arrow) needed to reach a prescribed goal. The meandering line represents a time series of data, and the circles represent a realized value (solid) and goal (open), respectively. (*b*) An example of an ecosystem-level reference point (RP; the smaller, stippled rectangle) or reference quadrat (RQ; dashed rectangle), control rule (CR), and direction (arrow) needed to reach a prescribed goal. The control rules may be based on either the reference point (CR(p)) or reference quadrat (CR(q)). The meandering line represents a time series of data, and the circles represent a realized value (solid) and goal (open), respectively.



The action (i.e., to produce movement from one quadrat to a more desirable quadrat, the vector of which we term the desired reference direction) could be primarily guided by using the canonical relationships established, focusing on those factors that we can control (i.e., human). This assumes the canonical relationships represent some form of dependent relationship among the metrics. For example, if the score were in the upper left quadrat (I) of Fig. 6 and we desired a groundfish-dominated community, the advice would be to not exceed landings of all groundfish beyond a total amount. That is, we would advise keeping the groundfish biomass consistent while maintaining consistent (but not escalating) effort. As another example, if the score were in the lower right quadrat (III) and one desired to remain in that quadrat, then the advice would be to preserve elasmobranch biomass and continue to fish other species groups, recognizing that environmental change may move the score into quadrats II or IV. There are numerous scenarios, and these are just examples of how one might use the reference quadrat and reference direction concepts as a multivariate analogue to single-species reference points and control rules.

The challenges of this approach are that even though it assumes dependent relationships, often the mechanism and specific processes for obtaining particular conditions are complicated or indeterminate. Additionally, even if we do understand specific mechanisms of causality, we may not be able to readily reproduce or manipulate them towards a desired end. For instance, we may have experienced a change of conditions such that the ecosystem is in an alternate steady state. It is possible that despite a clear reference direction, the multivariate trajectory will meander along an infinite combination of possible paths to reach the desired condition. Or it is possible that we may simply not be able to extend our multivariate trajectory in a direction back to the conditions observed in the 1960s to early 1970s without a change in temperature or the NAO. This observation has rather large implications for fisheries management (sensu Beamish 1993).

Another limitation of this approach is that although we may be able to establish multivariate ecosystem reference points or quadrats and determine the general multivariate direction to obtain a desired ecosystem goal, we lack the tools to prescribe the magnitude of how much we would need to change the system. Said another way, we know where the arrow (i.e., reference direction) should point (in multivariate phase–space), we just do not know how big it should be.

We show an example of common single-species reference points and an associated control rule (Fig. 8*a*). As an analogue, the same is shown at the ecosystem level (Fig. 8*b*). Both have points of reference in phase–space, implied limits via the control rules, and a suggested direction (arrows between the circles) to reach the prescribed goal. In the single-species case, if the value of the observed score (solid circle) is outside of the acceptable points of reference (e.g., too high a fishing mortality or effort or catch; too low a biomass or spawning stock biomass or abundance, etc.), then a control rule is invoked based on an agreed-upon set of criteria (e.g., overfishing is deemed to be occurring, the stock is deemed to be overfished, etc.). Appropriate action (e.g., reduce effort) to mitigate the observed condition into a more desirable condition (open circle) is taken, or at least recommended. In the multivariate ecosystem case, the reference points are more arbitrary and could be established as a certain fraction of the PC axes (e.g., less than 0.5 units on PC1, only positive scores on PC2; the smaller stippled rectangle), or could be designated as an entire quadrat (reference quadrat; the dashed rectangle). These could also correspond to something similar to the three different "stanzas" (i.e., ellipses) observed in Fig. 6. As in the single-species example, if the value of the observed score (solid circle) is outside of the acceptable range of the points of reference (or reference quadrat), then a control rule could be invoked to move the score into a more desirable condition (open circle). This particular example demonstrates the desired movement of the observed score in the upper-right quadrat to within the range of conditions bounded by the reference (upper-left) quadrat. The challenges are to develop less arbitrary ecosystem reference points and to develop the most appropriate multivariate action(s) for the control rules associated with these ecosystem reference points.

Recommendations

Although they exist, multivariate ecosystem points of reference do not necessarily suggest appropriate ecosystem control rules. Are ecosystem control rules feasible and practical?

We need to develop mechanistic or analytical models (and associated calculi) for key ecosystem processes. As described earlier, an example of a common single-species reference point and control rule is shown in Fig. 8*a*, whereas Fig. 8*b* shows the same at the ecosystem level. Both have a point of reference in phase–space, implied limits via the control rules, and directions to reach the prescribed goal, but we need a less arbitrary set of control rules for the ecosystem case. The single-species control rule is based on a deterministic understanding of a key underlying process (e.g., stock–recruitment relationships), whereas the ecosystem control rule in this example is entirely empirical. Underlying theoretical mechanisms are available for many of the individual processes in marine ecosystems. For example, ocean circulation models or predation theory or single-species models are reasonably well developed. Linking the various submodels together across disciplines such that we can have a standard, overriding ecosystem model that is less dependent on strict empiricism merits further examination. Using the results of the empirical and statistical approaches may help to conceive and parameterize analytical models (sensu Rigler 1982). However, we need to admit that it may be that we simply will not be able to fully develop an analogous model at the ecosystem level.

Single-species stock assessment (Hilborn and Walters 1992; Quinn and Deriso 1999), environmental impact assessment (Canter 2001), and ecological risk assessment (Suter 1993) have the requisite machinery and tools to provide appropriate management decision criteria. Lessons from these disciplines should be applied for the assessment and management of large marine ecosystems. The distinction between fisheries stock assessment and the environmental assessments is an optimization vs. risk averse set of approaches. Perhaps fisheries science would be wise to give extra consideration to the latter approach. A key challenge for ecosystem based fisheries management is determining how to simultaneously optimize multiple goals when optimization may be a risk-prone perspective.

Additionally, we need to further explore empirical and statistical methods. We propose contrasting predicted quadrat location derived from these multivariate methods with observed values to validate predictions from this approach. This should and can be done for many ecosystems. It should also be done for multiple scenarios within an ecosystem. Pace (2001) notes that even an empirically predictive approach is essential for assessing major environmental concerns. The role of empiricism has been criticized, but it has also demonstrated its value over the long term (Rigler 1982; Peters 1986). Other existing (e.g., redundancy analysis, multidimensional scaling, canonical correspondence analysis, etc.) and yet-to-be-developed multivariate tools should be explored for continuing this approach and to provide even further insight into the interpretation of ecosystem status. We conclude that (*i*) a suite of metrics is required to present the composite picture of the status of an ecosystem, and focusing on just a limited few may be misleading, (*ii*) assessing marine ecosystems is feasible now, (*iii*) multivariate points of reference at the ecosystem level exist, and (*iv*) we may never develop control rules that detail the magnitude of required changes, but the concept of reference directions may be useful for ecosystem-based fisheries management. Empirical and statistical approaches may only be able to give us reference direction, but that may be sufficient for management at the ecosystem level.

Finally, developing the management criteria, theoretical basis, and philosophical acceptance thereof in fisheries sciences remains a key challenge. If fisheries managers and other stakeholders can be more informed of overall ecosystem status and fisheries assessments from a more holistic perspective, then they would be better equipped to choose actions that are more sustainable for the long term (Pitcher 2001; Sainsbury and Sumaila 2001). We hope that the information provided in this work serves as a useful beginning for the difficult task of operationalizing ecosystem-based fisheries management.

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Controls on fish distribution and abundance in temporary wetlands

Matthew J. Baber, Daniel L. Childers, Kimberly J. Babbitt, and David H. Anderson

Abstract: Our main goal was to determine if fish distribution and adundance in temporary wetlands were shaped primarily by large-scale (landscape) or small-scale (local) characteristics and to investigate the influence of cattle ranching on fish assemblages. A total of 24 temporary ponds were selected at the Kissimmee Prairie Sanctuary and the MacArthur Agro-Ecology Research Center. Each wetland was sampled for fish using throw traps and dip nets during 1999. Landscape processes (connectivity to permanent water bodies) predominately influenced fish assemblages, although local processes (depth–hydroperiod) were also important. Furthermore, no colonizing species went locally extinct before wetlands began to dry. Our findings suggest that large-scale processes that influence colonization dynamics are of more importance than small-scale processes that influence extinction dynamics. Finally, hydrological changes (ditching) associated with agriculture (cattle ranching) have adversely affected temporary wetland fish assemblages by reducing wetland hydroperiods and connectivity.

Résumé : Les objectifs principaux de notre étude sont de déterminer si les populations de poissons qui habitent les terres humides intermittentes sont affectées surtout par les facteurs à grande échelle (paysage) ou à petite échelle (milieu local) et d'analyser l'effet de l'élevage de bétail sur les communautés de poissons. Au total, 24 étangs temporaires ont été choisis dans le sanctuaire de prairie de Kissimmee et le centre de recherche agro-écologique MacArthur. Les poissons de chaque étang ont été échantillonnés à l'aide de pièges-quadrats et d'épuisettes en 1999. Les caractéristiques du paysage (contact avec des nappes d'eau permanentes) sont les facteurs qui affectent le plus les communautés de poissons, mais les facteurs locaux (profondeur–durée de l'inondation) sont aussi importants. De plus, aucune espèce colonisatrice n'est disparue localement avant que les terres humides n'aient commencé à s'assécher. Nos résultats montrent que les processus à grande échelle qui affectent la dynamique de la colonisation sont plus importants que les processus à petite échelle qui régissent la dynamique de l'extinction. Enfin, les changements hydrologiques (creusage de fossés) associés à l'agriculture (élevage de bétail) ont un effet négatif sur les communautés de poissons des terres humides intermittentes en réduisant la période d'inondation et en diminuant les contacts entre les nappes d'eau.

[Traduit par la Rédaction]

Introduction

Recent conceptual developments in freshwater fish ecology have emphasized the need to consider ecological processes at multiple scales (Grossman et al. 1998 Angermeier and Winston 1998). This insight is especially relevant in highly variable systems such as temporary ponds. In these habitats, colonization by fish may depend heavily on large-scale processes (landscape characteristics), such as the frequency and duration of hydrologic connections to water bodies that contain source populations of fish (Snodgrass et al. 1996; Taylor 1997). These large-scale processes are likely to influence fish assemblages by interacting with species-specific dispersal abilities and lead to differential colonization probabilities among species (Lomolino 1994). Once fish have dispersed to a wetland, the likelihood of establishing self-sustaining populations depends on the number of colonists and, perhaps most importantly, on the extent to which ecological requirements are met (Loftus and Kushlan 1987).

Fish rarely occur in temporary aquatic environments because these habitats are subject to seasonal drying, and thus, most species are unable to colonize or withstand the harsh abiotic conditions (e.g., low oxygen levels, low conductivity, extreme temperatures, drying) associated with temporary wetlands (Loftus and Kushlan 1987). Studies to date suggest that fish presence in temporary wetlands ranges from 0 to 21% (e.g., Snodgrass et al. 1996). Despite the difficulties, colonizing fish may benefit from abundant food resources (e.g., tadpoles) and few potential competitors or predators (Snodgrass et al. 1996; Corti et al. 1999), at least until wetlands drawdown (Kushlan 1976).

The major factors and the relative importance of landscape and local characteristics influencing fish assemblages in temporary wetlands are unclear, largely because of a pau-

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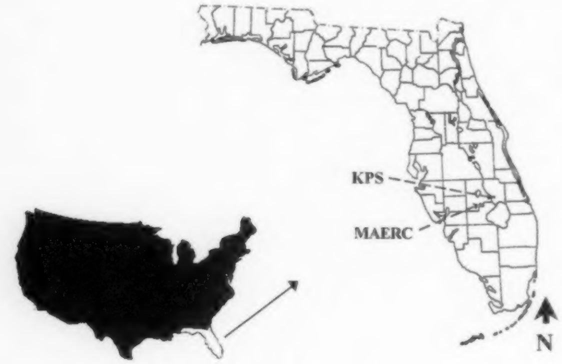
Fig. 1. Map depicting the geographic location of the MacArthur Agro-Ecology Research Center (MAERC, 27°20′N, 81°20′W) and the Kissimmee Prairie Sanctuary (KPS, 27°60′N, 81°10′W) in southcentral Florida, U.S.A. Study sites are approximately 80 km apart. Inset map of U.S.A. highlights the state of Florida.



city of studies (but see Snodgrass et al. 1996). At the landscape level, connectivity to other water bodies and the number of wetlands in the landscape are known to positively influence fish diversity in temporary aquatic systems (Snodgrass et al. 1996; Taylor 1997). However, fish assemblages are also known to be influenced by local parameters such as hydroperiod, which is considered a local characteristic when wetlands are filled predominately by precipitation (Snodgrass et al. 1996), wetland size (Angermeier and Schlosser 1989), dissolved oxygen levels (Brezonik et al. 1993), water chemistry (Dunson et al. 1997), habitat complexity (Chick and McIvor 1994), and predation (Rozas and Odum 1988).

Our goal was to quantify fish assemblages in temporary ponds in southcentral Florida, U.S.A., and to investigate which environmental factors predominately influence these assemblages. Specifically, we investigated the relative influence of landscape vs. local conditions on fish assemblages. This enabled us to identify if fish in temporary wetlands were influenced more by colonization processes or by a failure to cope with the harsh abiotic conditions associated with these habitats (as indicated by local extinction before wetland drawdowns). Hydroperiod and the degree of connectivity to other water bodies were expected to predominately influence species richness and abundance, because these processes are limiting in temporary wetland systems. Furthermore, in southcentral Florida, much of the historic habitat modification has resulted from drainage of vast areas of marsh and prairies to improve agricultural output. To examine the influence of anthropogenic disturbance on fish dynamics in this region, we compared temporary wetland fish assemblages in a modified agricultural landscape (cattle ranch) with a relatively unmodified native prairie landscape (reference site). The implications of these changes to fish assemblages in temporary ponds is unclear and, to our knowledge, has not been examined. Finally, we examined the similarities of these fish assemblages to those in nearby recruitment sources (permanent water bodies) to determine if these assemblages were random or nested subsets of their associated source pools. Nestedness is the tendency of smaller faunas to contain a subset of the species in the larger faunas (Patterson and Atmar 1986).

Materials and methods

Study area

Research was conducted in a native prairie landscape and a historically similar but modified agricultural landscape to include a range of habitat types and anthropogenic disturbances. Both of these sites occur within the Istokpoga Indian Prairie Basin, a formerly extensive wetland that once connected Lake Istokpoga to Lake Okeechobee in central Florida, and are approximately 80 km apart. This basin is located in the northern portion of the Greater Everglades region, which runs from south of the Kissimmee lakes to Florida Bay.

The agricultural study site was the MacArthur Agro-Ecology Research Station (MAERC, Fig. 1; 27°20′N, 81°20′W), a 4086-ha cattle ranch located approximately 22 km southeast of Lake Placid, Highlands County, in southcentral Florida. The ranch consists of improved pasture (pasture) and semi-improved pasture (semi-native prairie) interspersed with temporary wetlands and oak – cabbage palm hammock (*Quercus virginiana* – *Sabal palmetto*). The ranch was ditched extensively in the 1960s to enhance drainage and contains approximately 500 isolated temporary wetlands ranging in size from 0.1 to 20 ha. These wetlands are typically shallow depressions in sand substrate with herbaceous vegetation that often forms concentric bands. Wetlands at MAERC typically contain two vegetation zones: a central deepwater zone dominated by thick-stemmed, herbaceous emergents such as *Pontederia cordata* and *Sagittaria lancifolia* and an outer zone with the dominant vegetation dependent on the surrounding habitat matrix. In improved pasture, the outer zone is dominated by *Juncus effusus*, in semi-improved pasture by St. Johns Wort (*Hyericum fasciculatum*), and in forested habitat by various sedges. All outer zones also contain lower-stature emergents such as *Bacopa caroliniana* and *Hydrochloa carolinensis*.

The native prairie study site was the National Audubon Kissimmee Prairie Sanctuary (KPS; 27°60′N, 81°10′W), which is located in Okeechobee County, southcentral Florida (Fig. 1). The KPS was considered similar to the MAERC landscape before agricultural modification, and we therefore used these wetlands as reference sites to gauge the impact of cattle ranching. The KPS is also part of the Istokpoga Indian Prairie Basin and is 3050 ha with approximately 80 seasonal ponds ranging in area from 0.25 to 20 ha. In addition to native prairie vegetation, the prairie includes many oak – cabbage palm hammocks. Wetlands within KPS also exhibit concentric zones of vegetation presumably related to hydroperiod. The center of most ponds typically consists of a *Pontederia cordata* – *Panicum hemitomon* (Maidencane) zone that grades into a zone of St. John's Wort accompanied by *Utricularia* spp., *Nymphoides aquatica*, *Eleocharis* spp., *Carex* spp., *Ranunculus* spp., and *Rhynchospora* spp.

Data collection

We sampled a total of 24 wetlands. Site selection was stratified to ensure that all areas and major upland habitats at MAERC and KPS were represented equally, and wetlands

Table 1. Description of landscape, local abiotic, and local biotic variables measured for MacArthur Agro-Ecology Research Center and Kissimmee Prairie Sanctuary study wetlands ($n = 24$).

Variable	Description	Mean ± SE	Range
Landscape			
AREA	Wetland size (ha)	1.62±0.41	0.1–7.2
%WET	% Wetland in 250-m radius	10.8±1.8	0–38
CONN	Days connected to permanent water	1±1.3	0–5
Local abiotic			
pH	Average pH	5.7±0.16	4.4–7.1
COND	Average conductivity	103±15	20–270
DEPTH	Average maximum depth (cm)	47.3±3.5	20–77
HYDRO*	Wetland inundation (months)	3.46±0.37	2–6
Local biotic			
HABCOM	Average % vegetation cover	52.4±3.67	19–79
INVDENS	Average density of individual predators (m^2)	2.31±0.34	0.5–7.8

Note: SE, standard error.

*Hydroperiod (HYDRO) is considered a local characteristic when temporary wetlands are predominately filled by precipitation and in the context of the influence on amphibian community dynamics.

were randomly selected within stratified blocks. Six wetlands were chosen from each of the three major habitat types at MAERC (i.e., improved pasture, semi-native prairie, and woodland habitat), and six native prairie wetlands were selected at KPS. Improved pasture wetlands are considered heavily impacted because they are intensively grazed and ditched and consist of monotypic stands of bahia grass (*Paspalum notatum*). Semi-native prairie and woodland wetlands at MAERC are less intensively ditched and only lightly grazed and are therefore considered moderately impacted. We only selected wetlands that were not directly connected to ditches (at MAERC).

We measured landscape and local environmental variables that we considered to be most important in influencing fish assemblages in temporary wetlands (Table 1). Wetland size and the proportion of wetland habitat in the surrounding landscape were measured using the MAERC and KPS geographic information system (GIS) and databases (Table 1). Hydroperiod was measured as the number of weeks that a wetland held standing water during the 1999 wet season, and each wetland was checked weekly for the presence or absence of standing water. Wetland connectivity was measured as the number of days that a wetland was connected to permanent water (via surface water or ditch). Maximum depth was determined from measurements taken during each sampling period using a metre stick. Water pH and conductivity were measured at the center of each wetland during each sampling period using a Watercheck pH–conductivity meter (Hanna Instruments, Laval, Que.). Maximum and minimum temperature and rainfall data were continuously monitored using meteorological stations at MAERC and KPS.

We sampled each of the 24 wetlands monthly for fish during periods of inundation throughout the 1999 wet season (June to October). Each wetland was sampled two to five times depending on the length of inundation. Fish were sampled using a 30 cm × 45 cm dip net and a $0.25\ m^2$ aluminum throw trap (Streever and Crisman 1993). For each wetland, the number of traps deployed and the time spent dip-netting depended on the estimated inundated area and number of vegetation zones at the time of sampling. We deployed 5 to 15 throw traps in each inundated vegetation zone within the wetland and spent 15–45 min dip-netting. If additional species were captured at the end of the allotted throw-trap deployments, we continued throw-trapping until five consecutive throw traps revealed no new species. This minimized the chance of sampling error (i.e., not capturing species present in the wetland). Similarly, we continued dip-netting past the predetermined time until no new species were captured for 10 min. Traps were deployed randomly within each zone but at least 10 m apart. Throw traps were pressed into the substrate, and the vegetation zone, water depth, and habitat complexity (total vegetation cover to the nearest 20%) were recorded. All vegetation within each throw trap was then removed, and fish were collected within the throw trap by dip-netting until 10 consecutive sweeps revealed no additional specimens. All captured fish were identified, counted, and released.

We determined fish species composition from source pools (permanent water bodies) at MAERC (D.L. Anderson, unpublished data) and KPS (Trexler 1995) and compared them with species composition in temporary ponds at MAERC and KPS, respectively. At MAERC, fish colonized temporary wetlands via overflow from ditches or surface water runoff from permanent and semipermanent ditches in the surrounding landscape. At the KPS, temporary wetlands were colonized via surface water runoff from larger, permanent isolated wetlands connected to the Kissimmee River floodplain. We do not have fish composition information for these permanent isolated wetlands, but we do know the fish composition for the Kissimmee River and its floodplain (Trexler 1995), which is connected directly to these permanent wetlands.

Data analyses

We used nonmetric multidimensional scaling (NMDS) with the Bray and Curtis (1957) dissimilarity metric on a fish density by wetland site matrix. This analysis is an ordination method based on ranked distances and is well suited to data that are nonnormal or are on an arbitrary, or otherwise questionable, scale (McCune and Mefford 1997). The lack of fit between matrices ("stress") is measured by a loss function that the procedure minimizes. We started the NMDS analysis with six ordination axes and eliminated axes that had small incremental reduction in stress. Two ordination axes were sufficient to achieve low values of stress (6.3). Subsequently, 10 two-dimensional ordinations were performed using 100 iterations each. The ordination with the lowest final stress was selected for use in further statistical analyses in this study. The ordination was rotated using the varimax method, with the rotation based on maximizing the relationship between axis 1 and the connectivity to permanent water bodies, which showed the strongest relationship

Table 2. Fish species found in temporary isolated wetlands during the 1999 wet season.

Species	Abbreviation
Family Poeciliidae	
Mosquitofish (*Gambusia holbrooki*)	*G.ho*
Least killifish (*Heterandria formosa*)	*H.fo*
Sailfin molly (*Poecilia latipinna*)	*P.la* (KPS)
Family Fundulidae	
Goldentop minnow (*Fundulus chrysotus*)	*F.ch*
Bandedtop minnow (*Fundulus cingulatus*)	*F.ci*
Linedtop minnow (*Fundulus lineolatus*)	*F.li* (KPS)
Bluefin killifish (*Lucania goodei*)	*L.go* (MAERC)
Family Clariidae	
Walking catfish (*Clarias batrachus*)*	*C.ba*
Family Cyprinodontidae	
Flagfish (*Jordanella floridae*)	*J.fl*
Family Centrarchidae	
Bluespotted sunfish (*Enneacanthus gloriosus*)	*E.gl* (KPS)
Warmouth (*Lepomis gulosus*)	*L.gu*
Family Elassomatidae	
Everglades pygmy sunfish (*Elassoma evergladei*)	*E.ev*
Family Callichthyidae	
Armored catfish (unknown genus)*	*A.ca* (KPS)
Family Cichlidae	
Black acara (*Cichlasoma bimaculatum*)*	*C.bi* (MAERC)
Family Lepisosteidae	
Florida gar (*Lepisosteus platyrhincus*)	*L.pl* (MAERC)

Note: * denotes exotic species. KPS represents species captured at the Kissimmee Prairie Sanctuary only; MAERC represents species captured at MacArthur Agro-Ecology Research Center only.

to ordination space. Furthermore, to test if fish assemblages differed among habitat types, we performed nonparametric Kruskal–Wallis tests on NMDS axes 1 and 2 scores.

We used generalized linear models (GLMs; McCullagh and Nelder 1989) to estimate the relative importance of landscape and local explanatory variables on fish assemblage parameters (species richness, abundance, and species densities). These models present a major advantage over classical multiple regression approaches in that they integrate data from different statistical distributions with the appropriate modeling of statistical error (e.g., normal as in multiple regression, binomial for presence or absence data, ordinal for classes of abundance, poisson or negative binomial for species or individual counts).

Before conducting GLMs, we transformed explanatory variables where necessary to improve normality and conducted a Spearmans rank-order correlation analysis to determine which variables were collinear. There were strong correlations between depth and hydroperiod only. We excluded hydroperiod from the model because this variable exhibited a smaller change in deviance than depth (based on single-variable models). The deviance value represents a measure of the amount of variation in the model that can be accounted for by that particular variable. Response variables (species densities) were best represented by a poisson regression and logarithmic-link function (McCullagh and Nelder 1989). The goodness-of-fit model was assessed by the deviance of the models, and the change in deviance was tested using a χ^2 test (McCullagh and Nelder 1989), with a 5% significance level. Nonparametric Kruskal–Wallis tests were conducted to determine if species richness and fish abundance differed among habitat types.

A principal component analysis (PCA) on the wetland site by environmental variable matrix was used to examine patterns and differences in wetland environmental conditions among habitats. PCA was considered an appropriate technique for environmental data because of the data form (i.e., there are no large blocks of zero counts). Two-way nonparametric Kruskal–Wallis tests were used to determine which environmental variables differed significantly among habitats.

The colonization of temporary wetlands by fish occurred on a temporal scale. Therefore, we performed repeated-measure analyses of variance (rmANOVAs) on average species densities at each monthly sampling interval to determine if fish assemblages (species richness and abundance) differed with respect to the length of inundation. Data were square root transformed when left skewed, log transformed when right skewed, arcsine transformed when bimodally skewed, and not transformed when normally distributed. Ordination analyses (NMDS and PCA) were conducted using PC-ORD for Windows (McCune and Mefford 1997). Repeated-measure ANOVAs, GLMs, and Kruskal–Wallis tests were conducted using SAS/STAT software, version 8.2 (SAS 2001).

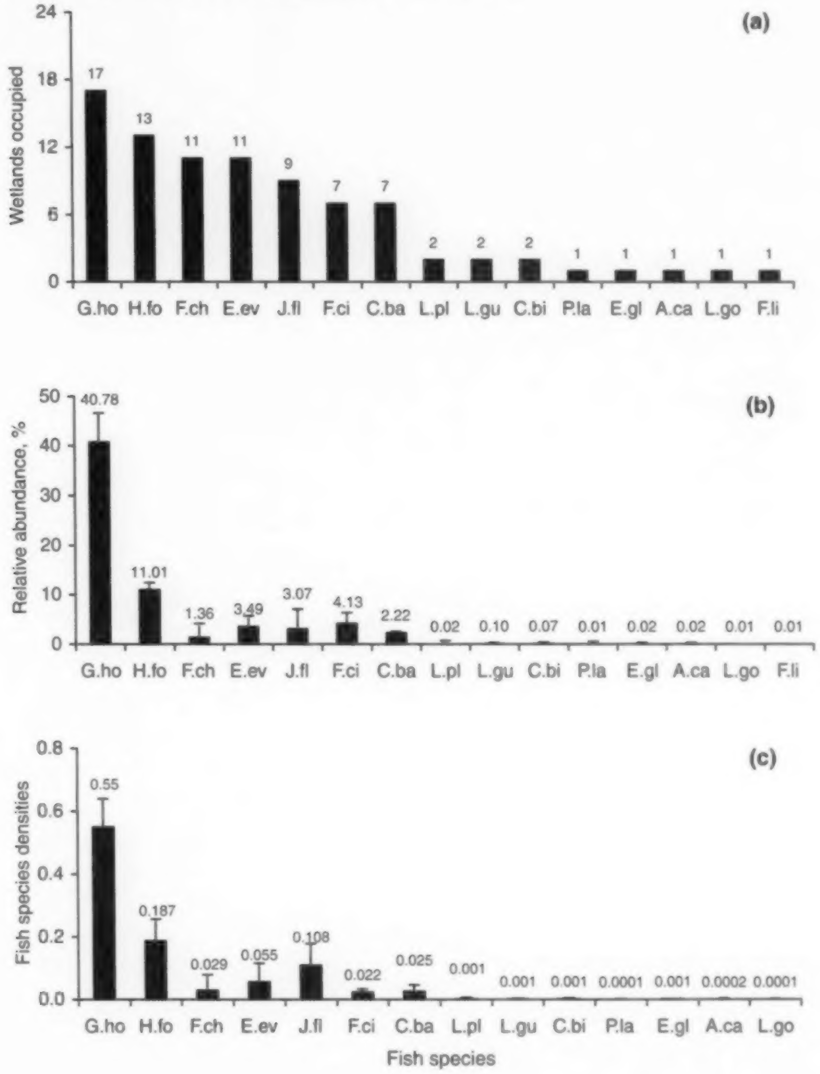
To test if temporary wetland fish assemblages at KPS and MAERC were nonrandom (nested) subsets of their respective source pools, we used the method described in Atmar and Patterson (1993). The metric used is *T*, the "matrix temperature", which ranges from 0° for a perfectly nested matrix to 100° for a matrix that is completely disordered. Statistical significance of the *T* value was assessed by a Monte Carlo simulation, using the "nestedness calculator" of Atmar and Patterson (1995).

Results

Fish assemblage description

Based on rainfall data collected over the last 20 years, the annual rainfall during 1999 (124 cm) was typical for the region. Fish colonized 71% (17 of 24) of the wetlands studied, and we captured a total of 3716 individuals representing 15 species (Table 2). Species richness averaged 4.3 ± 1.89 (mean ± standard error) and ranged from zero to 10 species per wetland. The most frequently occurring species was *Gambusia holbrooki*, which was found in all 17 of the wetlands containing fish. The least frequently occurring species were *Fundulus lineolatus*, *Lucania goodei*, and *Enneacanthus gloriosus*, *Poecilia latipinna*, and an unknown species of introduced armored catfish (escaped), which were found in only one wetland (Fig. 2*a*). Mean fish abundance within wetlands was $0.99 \pm 1.04\ \mathrm{m}^{-2}$ and ranged from 0 to $3.22\ \mathrm{m}^{-2}$. *Gambusia holbrooki* attained the highest relative abundance at 56.2%, and *L. goodei* had the lowest relative abundance of 0.00027% (Fig. 2*b*). Similarily, *G. holbrooki* attained the highest mean density of $0.776 \pm 0.87\ \mathrm{m}^{-2}$, whereas *L. goodei* had the lowest mean density of $0.0024 \pm 0.025\ \mathrm{m}^{-2}$ (Fig. 2*c*).

Fig. 2. (*a*) Number of wetlands occupied, (*b*) relative abundance (%) of species in temporary wetlands, and (*c*) density (no.·m^{-2}; mean density excluding unoccupied wetlands) during 1999. Species abbreviations: G.ho, *Gambusia holbrooki*; H.fo, *Heterandria formosa*; F.ch, *Fundulus chrysotus*; E.ev, *Elassoma evergladei*; J.fl, *Jordanella floridae*; F.ci, *F. cingulatus*; C.ba, *Clarias batrachus*; L.pl, *Lepisosteus platyrhincus*; L.gu, *Lepomis gulosus*; C.bi, *Cichlasoma bimaculatum*; P.la, *Poecilia latipinna*; E.gl, *Enneacanthus gloriosus*; A.ca, armored catfish (unknown genus); L.go, *Lucania goodei*; F.li, *F. lineolatus*.



Influence of environmental variables on fish assemblages

An NMDS revealed distinct, ecologically interpretable patterns of assemblage structure (Fig. 3). The first NMDS axis accounted for 70.7% of the variation in fish assemblage structure and represented a gradient from wetlands containing no fish to wetlands containing high species richness and densities of fish. Fishless wetlands were isolated from permanent water bodies, tended to have short hydroperiods, and were surrounded by a high proportion of upland habitat. Conversely, wetlands with high species richness and fish densities were often connected to permanent water bodies, had longer hydroperiods, and were surrounded by other wetland habitats. The second NMDS axis only accounted for 4.7% of separated wetlands and thus was not interpreted.

GLMs indicated that overall, fish assemblage parameters and species densities were most strongly influenced by landscape variables; however, local wetland conditions were still important. Fish species richness and abundance were most strongly influenced by connectivity, followed by depth–hydroperiod in that species richness and abundance was higher in deeper (and longer hydroperiod) wetlands connected to permanent water bodies (Table 3). Fish species richness was also influenced by the proportion of wetland

Fig. 3. Nonmetric multidimensional scaling (NMDS) based on a fish density matrix of wetlands at MacArthur Agro-Ecology Research Center (MAERC) and Kissimmee Prairie Sanctuary (KPS). Figure shows variation in fish assemblages among prairie (KPS, circles), pasture (MAERC, squares), semi-native prairie (MAERC, diamonds), and woodland (MAERC, triangles) habitats and the major environmental factors influencing community structure. R^2 cutoff = 0.2. Variable abbreviations: Wet, % wetland in 250-m radius; Depth, average maximum depth (cm); Hydro, wetland inundation (months); Conn, days connected to permanent water.

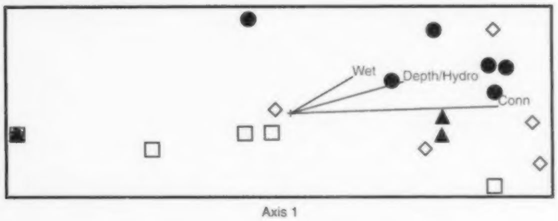


Table 3. Generalized linear models (GLMs) used to determine the significance of relationships between response variables (tadpole community and species parameters) and explanatory (environmental) variables.

Response variable	Explanatory variable	Deviance	χ^2	P
Species richness	CONN	82.8	23.2	<0.0001
	DEPTH	64.9	17.7	<0.0001
	WET	12.8	7.0	0.0081
Fish abundance	CONN	61.0	14.4	<0.0001
	DEPTH	43.0	9.7	0.0019
Gambusia holbrooki	CONN	3.8	6.9	<0.0081
Heterandria formosa	DEPTH	54.3	14.6	<0.0001
	CONN	12.1	7.6	0.0060
Fundulus chrysotus	DEPTH	57.6	16.4	<0.0001
	CONN	12.3	7.4	0.0067

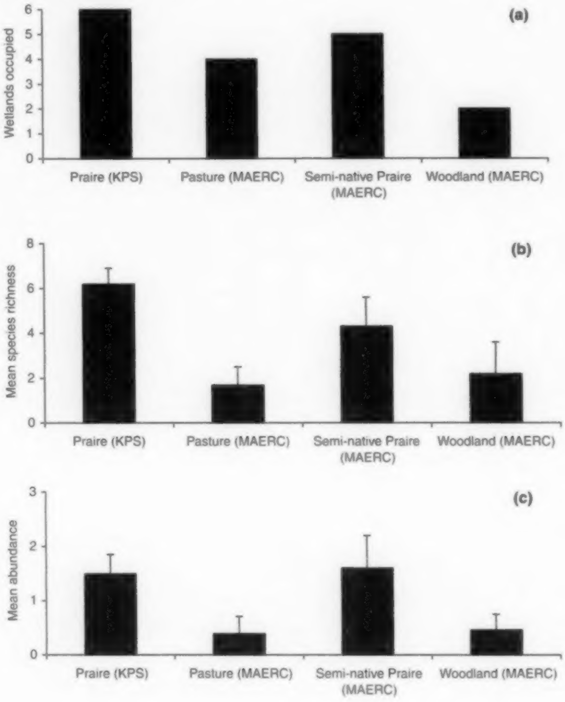
Note: Only significant relationships ($P < 0.01$) are presented. For abbreviations see Table 1.

habitat in the surrounding landscape. Connectivity and depth–hydroperiod also influenced densities of several species. Most importantly, of the seven species analyzed, three were significantly influenced by connectivity, and two were influenced by depth–hydroperiod (Table 3).

Habitat differences

The number of wetlands occupied by fish was highest in wetlands surrounded by native prairie habitat and lowest in wetlands surrounded by woodland habitat (Fig. 4*a*). Fish species richness did not differ significantly among habitats but did tend to be higher in native prairie wetlands (KPS; Fig. 4*b*). Likewise, fish abundance did not differ significantly among habitats but tended to be higher in native and semi-native prairie habitats than in pasture and woodland habitats (Fig. 4*c*). Overall, fish densities differed among habitat site scores along the first NMDS axis (H = 6.9, P = 0.036). Prairie and semi-native wetlands were more likely to have fish and attained higher fish densities than pasture and woodland wetlands. Fish densities did not differ significantly among habitats along the second axis.

Fig. 4. Habitat type vs. (*a*) number of wetlands occupied (n = 6), (*b*) mean species richness, and (*c*) mean abundance (no.·m^{-2}) during 1999.



PCA of environmental variables revealed distinct differences in environmental conditions among habitats, which largely explains why fish assemblages differ among habitats (Fig. 5). The first PC axis accounted for 94.6% of the variance in environmental conditions among wetlands and represented a gradient from shallow wetlands with short hydroperiods to deep wetlands with relatively long hydroperiods. In doing so, the first PC axis separated KPS wetlands from MAERC wetlands. Environmental conditions differed significantly among habitats along the first PC axis (H = 13.2, P = 0.003; Table 4). Prairie (KPS) wetlands were deeper with longer hydroperiods and lower habitat complexity than MAERC wetlands. The second PC axis accounted for only 4.4% of the variation, and environmental conditions among habitats did not differ.

Colonization dynamics

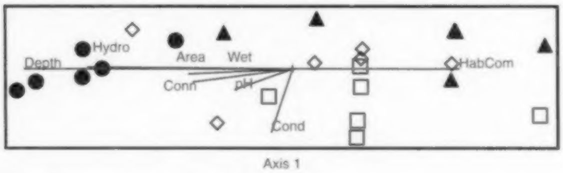
The proportion of wetlands colonized by fish increased rapidly with respect to the length of inundation, up to 10–12 weeks, and then leveled off (Fig. 6*a*). An rmANOVA revealed that the number of fish species also increased significantly with respect to wetland inundation (F = 22.4, P < 0.001; Fig. 6*b*), but the rate of increase slowed after 6–8 weeks. Similarly, the abundance of fish increased with wetland inundation (rmANOVA, F = 4.44, P = 0.014; Fig. 6*c*) up to 10–12 weeks and then decreased slightly.

Table 4. Kruskal–Wallis nonparametric tests for significance of differences in environmental variables among habitats.

	MAERC			KPS	
Environmental variable	Pasture, mean ± SE	SN prairie, mean ± SE	Woodland, mean ± SE	Prairie, x ± SE	P value
Landscape					
AREA (ha)	1.6±0.80	1.0±0.44	0.47±0.16	3.4±1.1	0.038*
WET (%)	5.7±1.7	9.7±2.2	7.2±2.1	20.8±4.2	0.015*
NPWET (m)	161±55	163±43	105±16	12.3±8.3	0.008**
CONN	1.1±1.3	3.2±1.9	0.3±0.4	4.16±1.8	0.036*
HYDRO (months)	2±0	3.0±0.63	3±0.62	4.83±1.7	0.001**
Local					
pH	5.9±0.23	5.7±0.37	5.2±0.15	6.1±0.36	0.168
COND	165±28	90±34	37±11	119±19	0.005**
DEPTH (cm)	38.3±4.0	45.8±5.1	35±5.2	70±2.4	0.003**
HABCOM (%)	63.0±5.0	62.0±1.2	35.0±5.2	24.3±1.6	0.003**

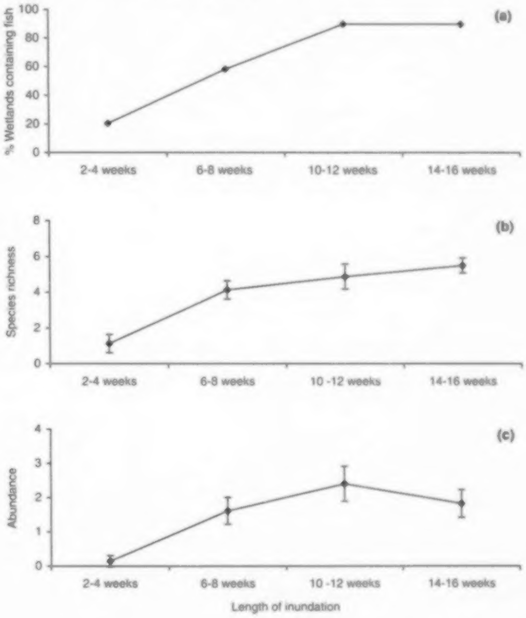
Note: SE, standard error; KPS, Kissimmee Prairie Sanctuary; MAERC, MacArthur Agro-Ecology Research Center; SN, semi-native prairie. NPWET, nearest permanent wetland; for other environmental variable abbreviations, see Table 1. *, $P < 0.05$; **, $P < 0.01$.

Fig. 5. Principle component analysis (PCA) based on a fish density matrix of wetlands at MacArthur Agro-Ecology Research Center (MAERC) and Kissimmee Prairie Sanctuary (MAERC). Figure shows the variation in environmental factors among prairie (KPS, circles), pasture (MAERC, squares), semi-native prairie (MAERC, diamonds), and woodland (MAERC, triangles) habitats. Prairie, pasture, and semi-native prairie habitat R^2 cutoff = 0.2. Variable abbreviations: Area, wetland size (ha); Wet, % wetland in 250-m radius; Depth, average maximum depth (cm); Hydro, wetland inundation (months); Conn, days connected to permanent water; pH, average pH; Cond, average conductivity; HabCom, average % vegetation cover.



The results from the presence–absence matrices clearly indicate that temporary wetlands at MAERC (T = 12.8°, $P < 0.001$) and KPS (T = 14.7°, $P < 0.001$) both represented distinct subsets of their respective source pools (MAERC canals and the Kissimmee River Basin). However, temporary wetlands at KPS contained three exotic species (*C. batrachus*, *C. bimaculatum*, and an armored catfish species, which escaped). None of these species was recorded in the Kissimmee River system (Trexler 1995). Furthermore, temporary wetlands at KPS and MAERC contained similar species.

Fig. 6. (*a*) Percent wetland colonized by fish, (*b*) fish species richness, and (*c*) fish abundance (no.·m^{-2}) with respect to the length of inundation during 1999.



Discussion

Fish assemblages in temporary wetlands of Florida

Fish colonized the majority of temporary wetlands studied (71%), and some wetlands had species-rich assemblages (up to 10 species). Our results differ markedly from studies in other regions, which have found temporary wetlands to either have depauperate fish assemblages or lack fish altogether (e.g., Moler and Franz 1987; Snodgrass et al. 1996). We attributed this to the ubiquity of isolated wetlands, the lack of topographic relief, and the life history characteristics of resident fish in the region. Before European settlement, wetlands occupied 55% of Florida's land cover and still comprised 32% of the area of the state by 1986 (Myers and Ewel 1990). The proportion of wetland habitat in the state is extremely high compared with the rest of the U.S. (5%) and with the southeastern U.S. (15%). Although a small portion of wetland area is comprised of temporary wetlands in Florida, these habitats may be numerically dominant in many parts of the state (Hart and Newman 1995). In addition, the lack of topographic relief increases the connectivity between water bodies by reducing elevation barriers and thereby aiding fish colonization.

Several fish species in Florida appear adapted to temporary wetlands (Hart and Newman 1995). *Gambusia holbrooki* is capable of dispersing between wetlands via surface water runoff during periods of heavy rainfall. Likewise, *C. batrachus*, introduced to Florida from Southeast Asia, has specialized gill structures and pectoral spines enabling overland dispersal during wet conditions (Rahman 1989). In addition, many species can tolerate low dissolved oxygen levels, some can survive in dormant wet muck (*J. floridae*), and some lay resting eggs (*F. chrysotus*) that hatch when wetlands become inundated again (Loftus and Kushlan 1987; Hart and Newman 1995).

Large- vs. small-scale environmental characteristics

Large-scale processes generally determine the potential species pool, whereas local processes such as predation and physical conditions tend to limit the number of species that coexist (Angermeier and Winston 1998). Fish assemblages in temporary wetlands of southcentral Florida are predominately influenced by processes operating at larger scales (connectivity), but local-scale processes are also important (depth–hydroperiod). In addition, we found no evidence of species extinction prior to wetlands drying up. Thus, our findings suggest that large-scale processes influencing dispersal, and not local conditions affecting the persistence of fish, predominately influence fish assemblages in temporary wetlands, at least until the onset of wetland drawdown. As such, based on our findings, fish assemblages inhabiting temporary wetlands may be largely driven by colonization processes, rather than by extinction. Nevertheless, the interplay between processes operating at multiple scales must be noted. Most importantly, the frequency of connections to permanent water bodies is heavily dependent on the hydroperiod of temporary wetlands. The influence of wetland connectivity on fish species richness in temporary or isolated wetlands has been reported in several studies (Kushlan 1976; Snodgrass et al. 1996; Poizat and Crivelli 1997). The longer and more frequent the connections to other water bodies, the greater the opportunity that fish species have to colonize a wetland (Poizat and Crivelli 1997). Depth–hydroperiod is also known to influence fish species richness because of the increased chance of colonization through time (Kushlan 1976; Snodgrass et al. 1996; Grossman et al. 1998). Fish abundance and species densities were also influenced by wetland connectivity and depth–hydroperiod, which may be attributed to the higher abundance of colonists in well-connected wetlands and the increased time to breed and populate in deeper wetlands with longer hydroperiods (Snodgrass et al. 1996).

Habitat differences

Clear differences in fish assemblages existed among habitats and reflected the hydrologic conditions of the surrounding landscape. Because of the higher connectivity among wetlands and longer hydroperiods, wetlands at the KPS attained higher fish species richness and densities and were rapidly colonized. In contrast, only 62% of wetlands at MAERC contained fish species, and fish assemblages typically had few species and low densities, particularly in pasture and woodland habitats. Pasture habitat in southcentral Florida is heavily ditched, which lowers the water table and reduces wetland hydroperiods. Woodland wetlands were not subjected to such intensive ditching; however, woodland habitat provided an effective barrier against fish colonization. The only two woodland wetlands in which fish occurred were in close proximity to a ditch that frequently overflowed.

Agricultural impacts

Agricultural systems in the greater Everglades region have typically been altered from a large, usually permanent, aquatic mosaic to a terrestrial system containing numerous isolated temporary wetlands with diverse hydrologic regimes (Babbitt and Tanner 2000). Thus, ditching has resulted in a decrease in the proportion of wetland habitat in the landscape. This is also supported by the higher connectivity and longer hydroperiods at the relatively unmodified KPS site. Because fish assemblages respond positively to longer hydroperiods and higher connectivity among wetlands, and because many wetlands at MAERC were isolated from ditches, it is likely that agricultural modification of this system has had a negative impact on fish assemblages.

Colonization dynamics

The proportion of wetlands containing fish, species richness, and abundance initially increased rapidly but then leveled off after wetlands were inundated for several months. In general, wetlands not colonized by fish in the first 2 months were unlikely to be colonized. All such wetlands were hydrologically isolated from potential source pools over the course of the study. The rapid increases during early periods may reflect the high connectivity of most wetlands early in the wet season or the abundance of high-quality food resources in temporary wetland systems relative to the number of consumers. The observed leveling-off that occurred after the first few months may be explained by an increase in intra- and inter-specific competition or by the observed increase in wading-bird predation as water levels decreased towards the end of the study (Loftus and Kushlan 1987). Several studies suggest that biotic interactions (competition and predation) play a stronger role in regulating populations in permanent aquatic habitats (Snodgrass et al. 1976; Corti et al. 1999).

Source pool assemblages

Most species found in source pools never colonized temporary wetlands, which may be explained by their preference for deeper water (Poizat and Crivelli 1997), their inability to cope with the highly variable conditions in temporary wetlands (Loftus and Kushlan 1987), or their relatively poor dispersal abilities. Moreover, fish assemblages in temporary wetlands clearly represented nested subsets (rather than distinct or random assemblages) of their respective source pools at both MAERC (ditches and canals) and KPS (permanent wetlands connected to the Kissimmee River Basin). This highlights that some species were better temporary wetland colonizers than others, for example, *G. holbrooki* was present in 71% of the study wetlands and attained the highest average densities ($0.55\ m^{-2}$).

Both KPS and MAERC temporary wetlands contained three exotic fish species. However, none of these species was known to occur in the Kissimmee River system (Trexler 1995). This implies that these exotics may be better adapted to conditions found in more disturbed water bodies in the region, such as ditches or canals. For example, the success of *C. batrachus* in Florida is largely attributed its ability to survive in poorer water quality than most species of native fishes (Taylor et al. 1984). Alternatively, it is possible that the Kissimmee River system species list was incomplete and that these wetlands received colonists from another source pool; the nearest ditch occurred off the KPS, approximately 200 m from the nearest study wetland and may have been indirectly connected.

Exotic species

Exotic fish predators are known to have detrimental impacts on temporary wetland organisms, particularly amphibians (M.J. Baber, D.L. Childers, K.J. Babbitt, and G.B. Noe, unpublished data). Despite the presence of this species in southcentral Florida for at least 25 years, this species does not appear to be common or abundant in temporary wetlands compared with native fish (e.g., *G. holbrooki* and *H. formosa*). Only 7 of the 24 wetlands studied contained *C. batrachus*, with an average abundance of 0.025 m^{-2}. In contrast, as many as 3703 $kg \cdot ha^{-1}$ of *C. batrachus* have been seined from some small natural ponds in Florida (Lachner et al. 1970), and thus the ecological impact of these species on native assemblages warrants concern. This species is known to consume invertebrates, fish, large amounts of detritus (Courtenay et al. 1974), and larval anurans (M.J. Baber and K.J. Babbitt, unpublished data)

In conclusion, temporary isolated wetlands in southcentral Florida typically support fish populations and, in some cases, species-rich fish assemblages. Our findings are in direct contrast with the findings for other regions and are attributed to the ubiquity of wetlands, the lack of topographic relief, and the ability of many fish to colonize these environments. Fish community structure was predominately influenced by large-scale processes (connectivity to permanent wetlands), but it was also influenced by processes operating at smaller scales (depth–hydroperiod). Furthermore, we found no evidence that colonized fish became extinct locally prior to wetlands drying. As such, colonization dynamics, rather than extinction dynamics, may control the distribution of fish assemblages in these systems. Differences in fish assemblages among habitats were due to hydrologic factors attributed to agricultural modification and topographic factors. Generally, agricultural conditions had a negative impact on temporary wetland fish assemblages.

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Depth-dependent variation in condition and length-at-age of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence

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Abstract: We describe depth-dependent variation in the condition and length-at-age of southern Gulf of St. Lawrence cod (*Gadus morhua*) on their feeding grounds in September 1971–2000. Bathymetric variation in condition appears to be linked to abundance. In periods of low abundance, condition was relatively uniform over shallow and intermediate depths (<100–125 m). During periods of high abundance, condition was highest in the shallowest waters and declined steadily as depth increased to 100 m. In all periods, condition was low in deep water. Bathymetric trends in length-at-age contrasted with those in condition. Length-at-age was high in the deep waters where condition was low. Length-at-age also tended to increase from minimum values at intermediate depths to high values in shallow waters. This tendency was most striking in the 1990s, a period when condition was uniform over this depth range. We discuss the ecological, bioenergetic, and sampling implications of these patterns.

Résumé : On trouvera ici une description de la variation en fonction de la profondeur de la condition physique et de la longueur aux différents âges de la morue franche (*Gadus morhua*) du sud du golfe du Saint-Laurent en septembre, de 1971 à 2000. La variation bathymétrique de la condition semble reliée à l'abondance. Durant les périodes de faible abondance, la condition est relativement uniforme dans les eaux peu profondes et intermédiaires (<100–125 m). Lorsque l'abondance est élevée, la condition est maximale dans les eaux les moins profondes et décline constamment jusqu'à une profondeur de 100 m. Durant toutes les périodes cependant, la condition est faible en eau profonde. Les tendances de la variation bathymétrique de la longueur aux différents âges diffèrent de celles de la condition physique. La longueur en fonction de l'âge est élevée en eau profonde où la condition est faible. La longueur tend à croître d'un minimum dans les eaux intermédiaires à des valeurs élevées dans les eaux peu profondes. Cette tendance est particulièrement marquée durant les années 1990, une période pendant laquelle la condition physique est uniforme à ces profondeurs. Les conséquences écologiques et bioénergétiques de ces patrons, ainsi que leur importance pour l'échantillonnage, font l'objet d'une discussion.

[Traduit par la Rédaction]

Introduction

Condition indices and length-at-age are important metrics of the productivity of fish populations. Besides the obvious negative impact on the yields that can be obtained from a population, poor condition of marine temperate fishes has been associated with lower reproductive output (Kjesbu et al. 1991; Lambert and Dutil 2000) and poor recruitment (Marshall and Frank 1999). Low condition and length-at-age also have been associated with increased natural mortality (Dutil and Lambert 2000; Sinclair 2001). Fish of poor condition and (or) low length-at-age can be indicative of adverse environmental conditions, poor feeding regime, or increased prevalence of parasites (Lambert and Dutil 1997*a*; Yaragina and Marshall 2000). Previous studies of condition and length-at-age have focused primarily on seasonal, interannual, and interpopulation comparisons. For example, the seasonal patterns in condition have been well documented for many cod (*Gadus morhua*) populations (Eliassen and Vahl 1982; Lambert and Dutil 1997*a*; Schwalme and Chouinard 1999). Studies of interannual variation in condition include the studies of Lilly (1996), Lloret and Rätz (2000), and Yaragina and Marshall (2000).

Length-at-age of Atlantic cod has declined significantly for a number of populations in the northwest Atlantic in recent decades. For example, the mean length of a 7-year-old cod from the southern Gulf of St. Lawrence declined from about 59 cm in the early 1970s to 49 cm in the early 1990s (Chouinard et al. 2001). The combined effects of density, size-selective fishing, and temperature are thought to be responsible for this decline (Sinclair et al. 2002). Cod in the southern Gulf of St. Lawrence population are among the slowest growing of all Atlantic cod stocks (Brander 1995).

The cod stock of the southern Gulf of St. Lawrence, like most cod populations in the northwest Atlantic, declined to low levels in the early 1990s, and abundance has remained low since then (Chouinard et al. 2001). During the main feeding season in summer and early fall, the stock occupies the shallow waters of the southern Gulf, where depths are mostly less than 100 m (Fig. 1). The diet of Atlantic cod is varied and depends on size. In the southern Gulf, young cod

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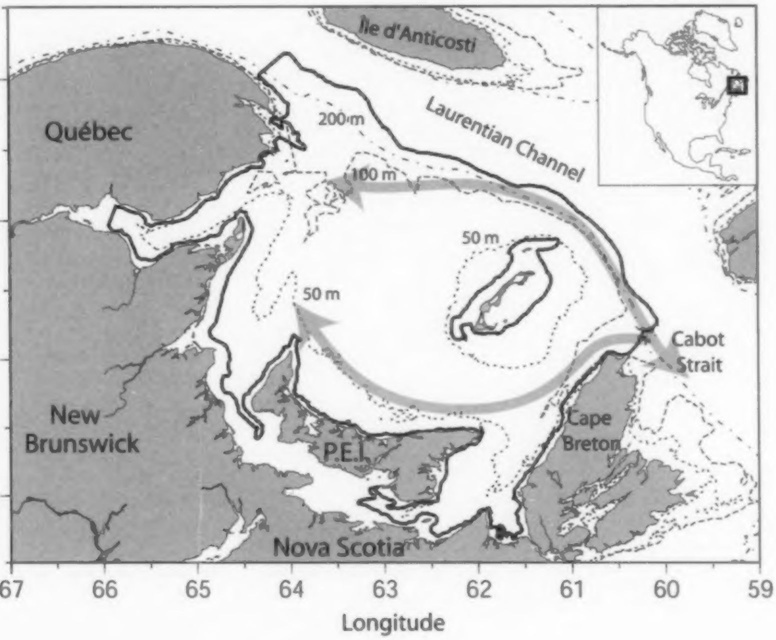
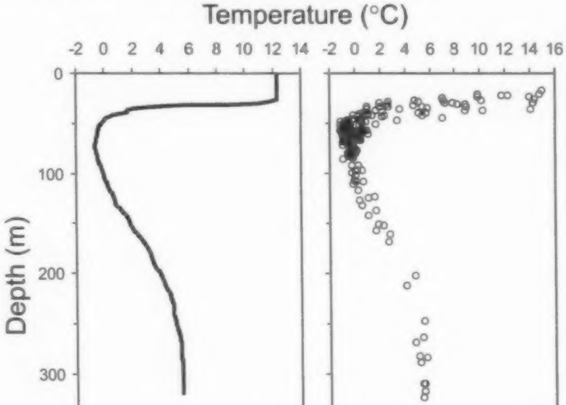
Fig. 1. Chart of the southern Gulf of St. Lawrence showing the 50-, 100-, and 200-m depth contours. Arrows show the annual migration pattern of cod (*Gadus morhua*) in the southern Gulf of St. Lawrence stock, and the thick solid line indicates the area covered by the bottom trawl survey.



(<30 cm in length) feed primarily on small crustaceans (Hanson and Chouinard 2002). Cod from 31 cm to 45 cm in length feed on larger invertebrates and small fish, such as capelin (*Mallotus villosus*) (Waiwood and Majkowski 1984). Finally, larger cod feed mostly on fish such as herring (*Clupea harengus*) and various flatfish species (Schwalme and Chouinard 1999; Hanson and Chouinard 2002). During summer and early fall, the water column in this area is composed of three layers (Koutitonsky and Bugden 1991): a warm surface layer, a cold intermediate layer, and a warm deep layer (e.g., Fig. 2). Late in the fall (October–December), southern Gulf cod migrate to the entrance of the Gulf in Cabot Strait where they overwinter in warm deep water along the slope of the Laurentian Channel (Swain et al. 1998). Little feeding occurs during this overwintering period (Schwalme and Chouinard 1999). The population returns to the southern Gulf in April–May, just before spawning. After spawning, the population disperses to feeding grounds throughout the southern Gulf.

Swain (1993) found that the bathymetric pattern of southern Gulf cod during the feeding season in September was related to their abundance. During periods of high abundance, densities tended to be highest at intermediate depths. However, in periods of low abundance, density was either unrelated to depth or highest in shallow water. It was hypothesized that these changes in the depth preference of cod reflected a trade-off between the density-dependent benefits of greater food supplies in warm, shallow waters and the density-independent benefits of lower metabolic costs in the colder waters at intermediate depths. It was suggested that cod occupied intermediate depths during periods of high abundance as a way of reducing metabolic costs when ration was low. Swain and Kramer (1995) found support for this hypothesis as cod occupied colder waters both in absolute and relative terms during periods of high abundance.

Fig. 2. Relationship between water temperature and depth in September in the southern Gulf of St. Lawrence: (*a*) a temperature profile taken on 14 September 1995 at 48°22′N, 62°03′W; and (*b*) bottom temperature versus depth at the sites sampled by the September 1995 trawl survey of the southern Gulf.



The purpose of this paper is to examine bathymetric variation in condition and length-at-age of cod during the feeding

Table 1. Summary of available data for the analysis of condition and size-at-age of cod (*Gadus morhua*) from annual research surveys in the southern Gulf of St. Lawrence.

		Length–weight measurements				Number of age-reading observations		
Year	Number of fishing stations	0–50 m[a]	51–100 m	101–150 m	>151 m	Age 4	Age 6	Age 8
1971	66	287	491	99	34	202	177	50
1972	70	429	342	189	82	335	173	100
1973	71	424	315	102	8	145	123	88
1974	64	413	328	86	35	226	147	49
1975	67	384	208	50	57	244	88	47
1976	66	306	344	91	28	245	97	28
1977	66	331	466	142	15	366	132	42
1978	63	487	441	188	52	428	100	23
1979	74	571	571	246	18	437	301	57
1980	70	549	714	280	7	285	367	70
1981	70	588	586	206	100	360	388	226
1982	67	350	640	192	37	71	59	63
1983	67	446	628	304	68	88	59	58
1984	108	854	849	241	92	61	52	60
1985	224	1423	1994	565	97	52	92	54
1986	173	951	1136	319	74	45	44	60
1987	162	985	1281	254	38	442	351	266
1988	154	879	1115	400	63	243	208	173
1989	169	1164	1350	261	61	502	500	227
1990	147	864	1163	251	101	448	326	169
1991	192	869	1391	210	88	642	354	217
1992	169	732	1050	174	85	492	324	80
1993	189	898	1141	226	89	476	520	123
1994	162	918	921	233	52	339	495	185
1995	182	1095	1039	162	131	428	332	365
1996	208	1191	1063	164	267	530	379	483
1997	214	1154	1063	247	105	381	413	281
1998	206	739	988	120	102	344	371	201
1999	193	746	877	138	49	399	183	168
2000	198	750	615	116	85	319	284	192

[a] Depth range. (m)

season in the southern Gulf of St. Lawrence. We interpret our results in relation to predictions from the ideal free distribution (IFD), a simple and robust hypothesis from behavioral ecology that predicts the distribution of foragers in relation to resources (Fretwell and Lucas 1970). If foragers have ideal knowledge of the environment, are free to move among areas, and have equal competitive abilities, the IFD predicts that foragers will be distributed among habitats so that foraging success will be equal in all occupied habitats. This prediction would result in spatially uniform condition and length-at-age. In contrast to this prediction, we find striking depth-related patterns in both condition and length-at-age. We discuss these patterns in relation to the assumptions of the IFD and describe their implications for sampling procedures to measure condition and for the development of bioenergetic models.

Methods

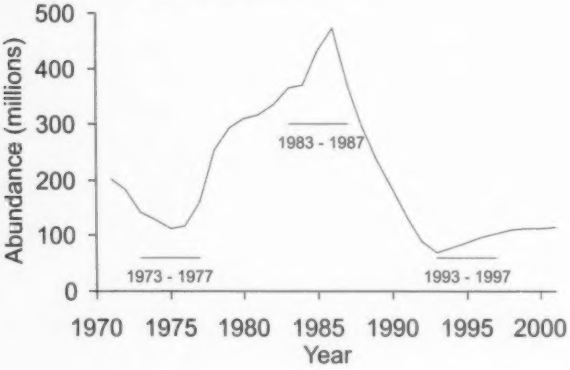
Individual observations of weight and length of cod have been routinely collected during the annual demersal fish survey conducted in the southern Gulf of St. Lawrence (Fig. 1) since 1971 by the Department of Fisheries and Oceans (DFO). This survey has always been conducted during the month of September, a time when cod are widely distributed in the area before their annual fall migration. Sampling stations were allocated over the survey area following a stratified random design (see Koeller (1981) and Hurlbut and Clay (1990) for details). The survey stations are located at depths ranging from 20 m to the deep waters (>200 m) of the southern edge of the Laurentian Channel (see Poirier et al. (2000) for a description of the 2000 survey). At each station, a 30-min fishing set was conducted using a bottom trawl fitted with a 19-mm liner in the codend. A Yankee 36 trawl was used in the period 1971–1985 and an Atlantic Western IIA was used in 1985–2000. (Note that both trawls were used in 1985.) Fork length (cm) was measured for all individuals. Otoliths were removed from length-stratified subsamples of the trawl catches for age determination. Fish in these subsamples were weighed with spring scales (±~10 g) from 1971 to 1989 and with electronic marine scales (±5 g) since 1990. Analyses of condition were restricted to fish from 30 cm to 60 cm in length because of low sample sizes at larger lengths and the difficulties in measuring weight of

Table 2. Results of the generalized additive model (GAM) analysis of the bathymetric variation in condition of cod (*Gadus morhua*) in the southern Gulf of St. Lawrence.

Effect	df	Deviance	*F*	*P*
(*a*) 1973–1977, N = 4133, residual df = 4123, residual deviance = 69.8				
Year	4	4.624	68.3	<0.0001
Depth	4	1.382	20.4	<0.0001
ln length	1	1465.309	8 6604.4	<0.0001
(*b*) 1983–1987, N = 12 599, residual df = 12 589, residual deviance = 268.8				
Year	4	18.736	219.4	<0.0001
Depth	4	10.261	120.2	<0.0001
ln length	1	4039.729	189 211.2	<0.0001
(*c*) 1993–1997, N = 12 159, residual df = 12 149, residual deviance = 115.7				
Year	4	1.225	32.2	<0.0001
Depth	4	2.898	76.1	<0.0001
ln length	1	3633.658	381 492.8	<0.0001

Note: df, degrees of freedom; *N*, sample size; *F*, the test statistic described by Venables and Ripley (1994, p. 187); *P*, significance level from this test.

Fig. 3. Abundance of age-3+ cod (*Gadus morhua*) in the southern Gulf of St. Lawrence stock. The short lines indicate the periods used in the generalized additive models.



small fish at sea with spring scales. Sampling intensity has increased over time, from about 70 stations and 700–1000 cod length–weight measurements per year in the early 1970s to about 200 stations and 1500–2500 length–weight measurements in recent years (Table 1).

Fulton's *K* ($K = W \cdot L^{-3}$) is often used as a measure of condition. However, the use of Fulton's *K* can be problematic because growth is not isometric in many fishes. In these cases, differences in condition indices like *K* can be artifacts of differences in the length distributions of the populations compared (Bolger and Connolly 1989). An alternative approach, which circumvents this problem, is to use predicted weight at a standard length (based on length–weight regressions) as an index of condition (e.g., Schwalme and Chouinard 1999). In this analysis, we have extended this approach, using generalized additive models (GAMs) to examine the bathymetric variation in condition of southern Gulf of St. Lawrence cod. GAMs are an extension of generalized linear models (GLMs) but have the advantage that the relationships between the dependent and independent variables are not constrained to particular parametric forms (Hastie and Tibshirani 1990). Instead, terms in a GAM can be modeled nonparametrically using a scatterplot smoother. Where a GLM assumes a linear form for the conditional expectation, a GAM, through its use of smooth functions, allows for nonlinear responses.

Condition was first examined in blocks of 5 years (1973–1977, 1983–1987, and 1993–1997) to determine if the bathymetric patterns were similar throughout the time series. These three time periods were chosen because they also corresponded to periods of low (1973–1977; 1993–1997) and high abundance (1983–1987). Abundance of the southern Gulf of St. Lawrence cod stock during the period of high abundance in the mid-1980s was about four times greater than in the periods of low abundance (Fig 3; Chouinard et al. 2001). Models used for this analysis were of the form:

$$\ln W_{ijk} = \beta_0 + \beta_1 \ln L_{ijk} + \beta_{2i} + s_1(D_j) + \varepsilon_{ijk}$$

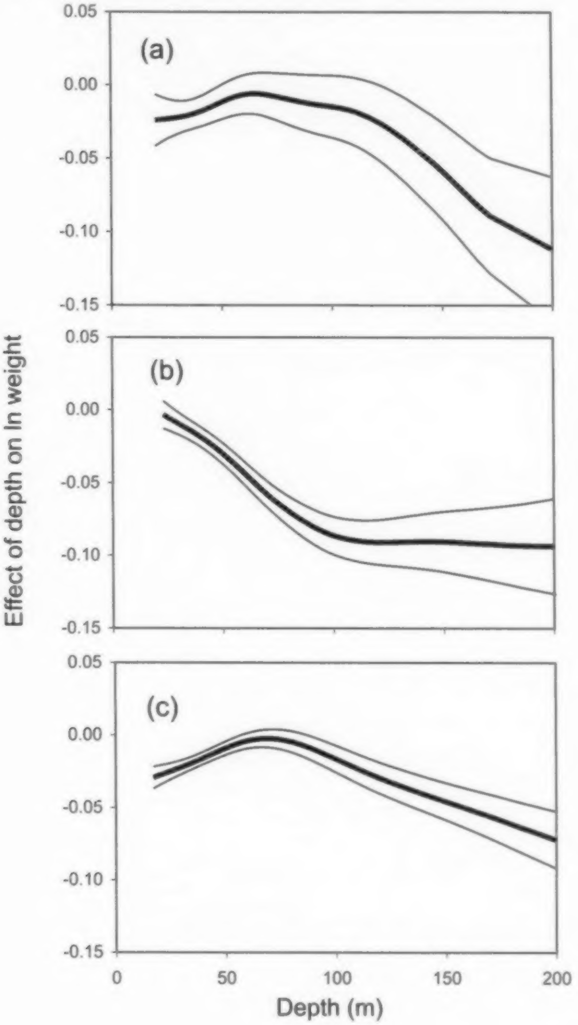
where $\ln W_{ijk}$ is the $\log_e$-transformed weight of fish k caught at depth j in year i, β_0 is the intercept, β_1 is the slope parameter for the effect of the $\log_e$-transformed fork length ($\ln L_{ijk}$) of this fish, β_{2i} is a vector of parameters for the class variable year, and $s_1(D_j)$ is a cubic spline function of depth. We specified the degree of smoothing for the depth term by setting the effective degrees of freedom for this term to 4.

We also conducted analyses using additional 5-year blocks within each of the abundance periods (e.g., 1992–1996 and 1994–1998 in addition to 1993–1997) as well as separate analyses for each year within each of the 5-year blocks. These analyses confirmed that results were not sensitive to the specific 5-year block chosen and indicated no strong interannual variation in the depth effect within the 1973–1977, 1983–1987, and 1993–1997 blocks. Thus, results are shown only for the original 5-year blocks.

A model incorporating age as a class variable was fit to the data as well. Although this effect was significant, the overall depth effect was similar to the models excluding age. Because sample sizes were greatly reduced for the 1983–1987 period when fewer fish were aged, only the models excluding age were considered.

We also examined patterns in condition as a function of depth and year over the entire 30-year time period. First, we fit the length–weight relationship over the entire dataset,

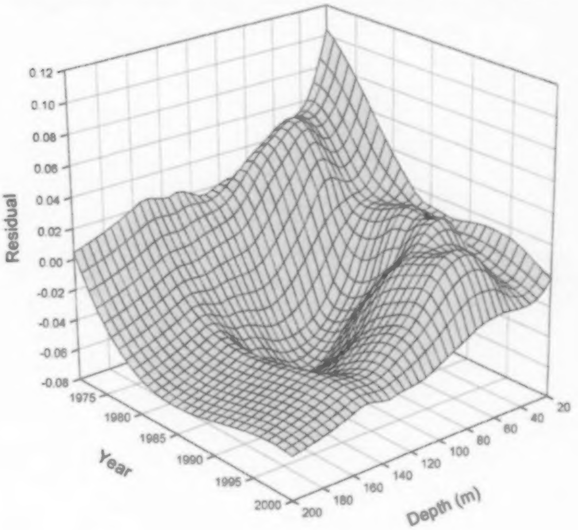
Fig. 4. Bathymetric trends and 95% confidence intervals for condition (ln weight) for three time periods ((*a*) 1973–1977, (*b*) 1983–1987, and (*c*) 1993–1997) for southern Gulf of St. Lawrence cod (*Gadus morhua*).



based on the linear regression of log weight as a function of log length, using a common slope and intercept for all years and depths. Residuals from this common length–weight relationship were then modeled as a function of year and depth (both treated as continuous variables in this analysis) using a local regression model (loess), a nonparametric method for estimating response surfaces (Cleveland 1993). Unlike in GAMs, there are no restrictions on relationships among predictors in loess models. We fit locally weighted linear functions ($\lambda = 1$) of the predictors over neighborhoods of 25% of the data (span = 0.25).

Length-at-age variation was also examined in the same blocks of 5 years for three age groups (4, 6, and 8 years) using GAMs. Models were of the form

Fig. 5. Loess response surface of condition for southern Gulf of St. Lawrence cod (*Gadus morhua*) by depth from 1971 to 2000.



$$L_{ijk} = \beta_0 + \beta_{1i} + s_1(D_j) + \varepsilon_{ijk}$$

where L_{ijk} is the fork length of fish k caught at depth j in year i, β_0 is the intercept, β_{1i} is a vector of parameters for the class variable year, and $s_1(D_j)$ is a cubic spline function of depth. The response surface of length-at-age as a function of year (treated as a continuous variable) and depth was also examined for the entire period using local regression ($\lambda = 1$, span = 0.25).

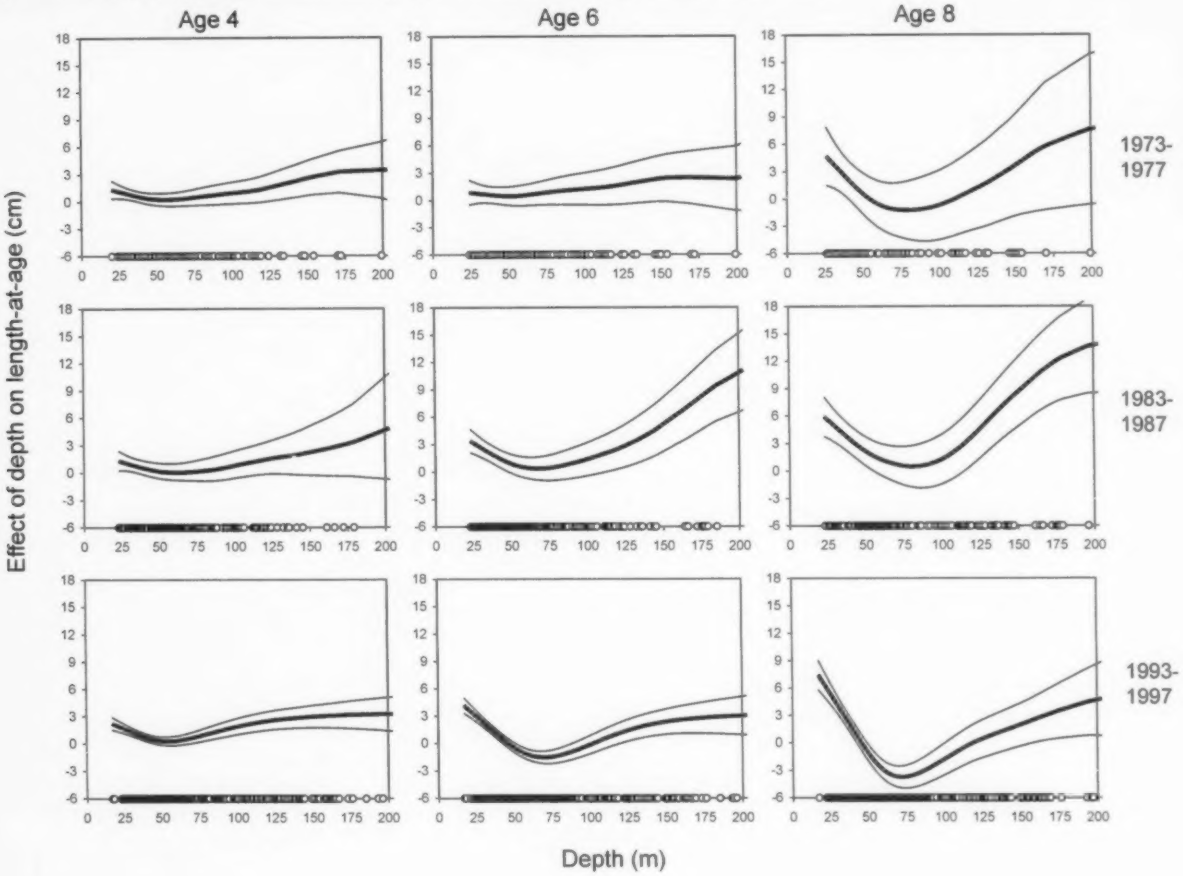
Results

Condition indices

Although most of the variation in weight was related to variation in length, a significant depth effect occurred in each of the three 5-year blocks (Table 2). This effect of depth on condition differed between the three periods (Fig. 4). In the low-abundance periods (1973–1977, 1993–1997), condition was high in shallow and intermediate depths but declined with increasing depth in the deeper waters (below 100–125 m). In contrast, during the high-abundance period in 1983–1987, although condition was also high in the shallowest waters, it declined sharply as depth increased from 20 m to 100 m and remained low in deep waters (>100 m).

Similar patterns are revealed by the loess surface of residuals from the length–weight relationship over the entire 30-year time series. This analysis also emphasizes the overall decline in condition from relatively high values in the early to mid-1970s to lower values throughout the 1980s and 1990s (Fig. 5). Over the whole surface, the condition index declined to a minimum at intermediate depths in the mid-1980s and recovered to moderate values at these intermediate depths in the 1990s. In the 1970s, the condition index was high at shallow and intermediate depths and lower in deep water, although the lower values in deep water in the

Fig. 6. Bathymetric trends and 95% confidence intervals in length-at-ages 4, 6, and 8 years for southern Gulf of St. Lawrence cod (*Gadus morhua*) in September in three time periods. Circles on the *x* axis indicate observations.



early 1970s were comparable to the highest values (at intermediate and (or) shallow depths) in the 1980s and 1990s. Variation in predicted condition among depths in a single year was often as great as the variation among years at a single depth.

Length-at-age

Bathymetric trends in length-at-age contrasted with those in condition. Length-at-age was high in the deep waters where condition was poor (compare Fig. 6 and Fig. 4). Length-at-age tended to be at a minimum at intermediate depths and high, like condition, in shallow water (Fig. 6). This tendency for length-at-age to decline from high values in shallow waters to a minimum at intermediate depths was strongest for older cod and in the 1993–1997 period. For the younger ages in the earlier periods, the effect of depth was smaller and the main feature of the bathymetric pattern was an increase in length in deep waters. Unlike the earlier periods when length tended to be greater in deep waters than in shallow waters, length-at-age tended to be as high in the shallowest waters as in the deep waters in the 1993–1997 period. Bathymetric trends in length-at-age tended to be sharpest (i.e., 95% confidence bands tended to be tightest) in the 1993–1997 period and weakest in the 1973–1977 period. The tighter confidence bands in the 1993–1997 period are partly attributable to greater sample sizes in this period (Table 1).

The loess response surfaces emphasize the overall decline in length-at-age between the early to mid-1970s and the mid- to late 1980s (Fig. 7). Length-at-age recovered somewhat in the 1990s in shallow waters but not at intermediate depths, where it remains near the minimum values in the 30-year time series. Unlike bathymetric patterns in condition, which differed between abundance periods, bathymetric patterns in length-at-age tended to be more consistent over the 30-year time period. This was especially true for older cod (age 8 years), which showed the same decline in length to minimum values at intermediate depths in all years. For younger cod, bathymetric patterns in length changed somewhat over the time series, with the decline to minimum values at intermediate depths strongly evident in recent years but only weakly evident or absent early in the time series. The surfaces also emphasize that bathymetric differences in length-at-age within a single year can be nearly as great as

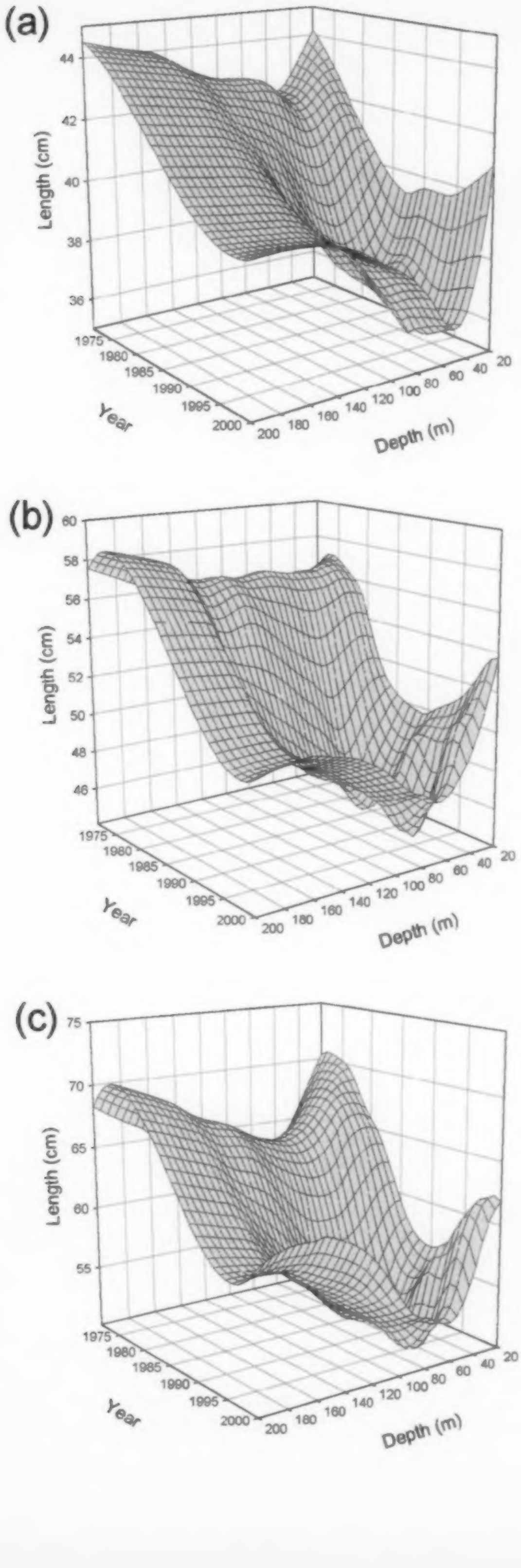


Fig. 7. Loess surface of length-at-age by depth and year for cod (*Gadus morhua*) in the southern Gulf of St. Lawrence: (*a*) age 4, (*b*) age 6, and (*c*) age 8.

the striking interannual variation over the 30-year time series. For example, predicted length at age 8 varied by about 10–16 cm over years at a single depth and by 6–12 cm over depths in a single year.

Discussion

Both length-at-age and condition of southern Gulf of St. Lawrence cod declined dramatically in the late 1970s and early 1980s. Causes of the decline in condition have not yet been examined in detail, but Sinclair et al. (2002) attributed the decline in length-at-age mainly to changes in size-selective mortality combined with the density-dependent effects of an increase in cod abundance. To the extent that length-at-age and condition reflect foraging success, the IFD predicts that interannual changes in these measures of growth should be spatially uniform. According to the IFD, cod would be expected to distribute themselves each year during the feeding season so that foraging success and growth rate would be uniform over all habitats. Instead, the bathymetric patterns reported here indicate considerable spatial structure in both condition and length-at-age of southern Gulf cod during their feeding season. This departure from IFD predictions could reflect variation in the competitive ability of southern Gulf cod, with poorer competitors displaced to poorer habitats.

When foragers are distributed according to the IFD, forager density "maps" intrinsic habitat quality. In our case, forager distribution departed from IFD predictions, and foraging success may provide an indication of habitat quality. Condition should be a better indicator of recent foraging success than length-at-age. In all years, condition was relatively low in deep water (>125 m). Cod densities also tended to be low in these deep waters (Swain 1993). This suggests that the deeper waters of the southern Gulf represent marginal habitat for cod in terms of food resources. Although detailed analyses of the distribution of food resources for cod in the southern Gulf have not yet been undertaken, many important food items for cod (e.g., herring; Poirier et al. 2000) are rarely found in these waters in September. These deep waters are also relatively warm, lying below the cold intermediate layer (CIL; Koutitonsky and Bugden 1991) so that metabolic costs are also relatively high in these areas. Thus, these areas appear to have poorer overall conditions for the accumulation of energy reserves in southern Gulf of St. Lawrence cod.

Condition at intermediate depths appeared to be density-dependent. During periods of low abundance, condition was high at both shallow and intermediate depths. At high abundance (i.e., in the 1980s), condition declined from high values in the shallowest water to low values at intermediate depths. This change in the bathymetric trend in condition coincided with a change in the depth distribution of cod. At low abundance, density of southern Gulf cod tended to be highest in shallow water (younger cod) or unrelated to depth (older cod), whereas at high abundance cod densities tended to be highest at intermediate depths (Swain 1993, 1999). Thus, during periods of low abundance, the IFD prediction of equalized profit rates holds over the shallow to intermediate depth range (at least in terms of condition), and forager density should map intrinsic habitat quality. The higher cod densities in shallow water at low population abundance suggest that intrinsic habitat quality is highest in shallow water. At high population abundance, the simple IFD predictions fail even over the shallow to intermediate depth range, as condition declines to low levels at the intermediate depths where cod densities are highest. This is also consistent with a decline in intrinsic habitat quality from shallow to intermediate depths, with the poorer competitors concentrating in the cold waters at intermediate depths to reduce metabolic costs when competition is high and food ration is low.

The bathymetric patterns in length-at-age suggest a high degree of spatial structure in the southern Gulf cod population on the feeding grounds. The patterns in length-at-age tended to be strongest for older ages. Because length-at-age reflects the growth history of an individual cod, the patterns observed in the southern Gulf population indicate that particular individuals tend to return to the same depth zones each year (though this tendency may be weakened by density-dependent changes in average depth preferences (Swain 1993)). Depth-dependent patterns in vertebral number of southern Gulf cod also suggest fine-scale spatial structure of cod on the feeding grounds in the southern Gulf (Swain and Frank 2000). This relationship between vertebral number and depth may result from mixing between two stocks, one with a preference for shallow waters and one with a preference for deep waters, or may reflect an association between morphology and habitat selection within a single population (Swain and Frank 2000).

Depth-dependent trends in vertebral number and length-at-age are not parallel. Mean vertebral number of southern Gulf cod increased linearly with depth, whereas length-at-age shows a nonlinear relationship with depth, with a minimum at intermediate depths. However, both patterns could reflect the same variation in depth preferences. Vertebral number is fixed early in life (before hatching in most fishes), so the bathymetric trend in vertebral number appears to reflect a correlation (possibly genetic) between the number of vertebrae formed in individuals and their subsequent depth preference, rather than differences in vertebral number induced by differences in depth distribution (see discussion in Swain and Frank (2000)). On the other hand, the bathymetric trend in length-at-age may reflect environmentally induced variation resulting from the differences in depth preference.

The bathymetric trend in length-at-age closely follows bathymetric variation in bottom temperature. In summer and early fall, temperature in the southern Gulf is high in shallow water, cold at intermediate depths, and relatively warm in deep water. Perhaps temperature affects how energy is apportioned between growth in length versus condition, with a higher proportion of energy apportioned to growth in length at higher temperatures. This would provide an explanation for the bathymetric trends in both length-at-age and condition. It also suggests an explanation for the stronger decline in length-at-age at intermediate depths in the 1990s, when temperatures at intermediate depths were unusually cold (Drinkwater et al. 2001). The patterns observed underline the importance of including distribution information in the development of bioenergetic models for this population.

Many authors (see Macpherson and Duarte (1991) and references therein) have documented a general trend for larger demersal fish to be found in deeper waters. Macpherson and Duarte (1991) concluded that this trend reflected a migratory movement towards deeper waters during ontogeny. Swain (1993) found a similar pattern for cod in the southern Gulf of St Lawrence; older (generally larger) fish tended to be found in deeper waters. The previous studies examined the bathymetric variation of either length or age independently. However, this study suggests that the observed pattern is likely more complex than previously thought because cod of all ages tended to be larger in deeper waters.

To our knowledge, bathymetric patterns in condition have not previously been reported. In recent years, a greater emphasis has been placed on monitoring fish condition as a measure of overall energy reserves (Lambert and Dutil 1997*b*). The patterns observed here indicate that variation in condition can have a significant spatial component. This spatial variation should be considered in the design of sampling programs to monitor interannual changes in condition. For example, in the case of the southern Gulf of St. Lawrence cod stock in September, designs that do not take spatial distribution into account may produce biased estimates of the variation in condition. Statistical sampling designs that allow for the calculation of depth-stratified abundance-weighted estimates of condition would be more appropriate for this purpose.

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Numerous scientific staff involved in the annual demersal research surveys in the southern Gulf of St. Lawrence over the span of 30 years collected the data used in these analyses. Their work, as well as the assistance of the crews of the research vessels *E.E. Prince*, *Lady Hammond*, and *CCGS Alfred Needler*, is gratefully acknowledged.

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Allozyme and microsatellite diversity in natural and domestic populations of turbot (*Scophthalmus maximus*) in comparison with other Pleuronectiformes

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Abstract: Twelve microsatellite and 28 allozyme loci were employed to analyse genetic diversity in natural and domestic populations of turbot (*Scophthalmus maximus*) from northwest Spain in comparison with other flatfish species with similar habitat, life history, and geographic distribution—the brill (*Scophthalmus rhombus*) and the flounder (*Platichthys flesus*). These species had shown much higher allozyme diversity than turbot in previous studies, and were used as a reference to check for putative historical bottlenecks in turbot. Significantly lower genetic variability in turbot than in brill and flounder was confirmed with allozymes, but not with the highly variable microsatellite loci. This intermarker discrepancy could be explained by different mutation rates in relation with historical bottlenecks along turbot evolution. A significantly lower genetic diversity was observed in a domestic strain of turbot than in natural populations of this species. This sample evidenced a strong family structure from microsatellite data, which suggests caution against the use of commercial batches for broodstock foundation in turbot farming. A strong concordance was found across the two categories of markers used when analysing the pattern of genetic subdivision at a local scale within the three species analysed, low and nonsignificant genetic differentiation being observed between Atlantic and Cantabric areas.

Résumé : L'étude de 12 sites microsatellites et de 28 locus d'allozymes nous a permis d'analyser la diversité génétique de populations naturelles et domestiquées du turbot (*Scophthalmus maximus*) du nord-ouest de l'Espagne et de la comparer à celle de deux autres poissons plats, la barbue (*Scophthalmus rhombus*) et le flet (*Platichthys flesus*), qui possèdent un habitat, un cycle biologique et une répartition géographique similaires. Lors d'études antérieures, ces espèces affichaient une diversité d'allozymes beaucoup plus élevée que le turbot; on les utilisait donc comme point de référence pour vérifier l'existence de présumés goulots d'étranglement dans l'histoire génétique du turbot. Nous pouvons, à l'étude des allozymes, confirmer la variabilité génétique réduite du turbot par comparaison avec la barbue et le flet, mais pas à l'examen des locus microsatellites qui sont très variables. Le désaccord entre les deux marqueurs peut sans doute s'expliquer par des taux de mutation différents en fonction des divers goulots d'étranglement durant l'évolution du turbot. La diversité génétique est significativement plus basse chez les lignées domestiquées de turbot que chez la population naturelle. Les données sur les microsatellites de notre échantillon révèlent l'existence d'une forte structure familiale; il y a donc lieu d'émettre une mise en garde contre l'utilisation de groupes commerciaux comme source des stocks reproducteurs dans l'élevage commercial des turbots. Les deux catégories de marqueurs montrent une forte concordance dans l'analyse des structures de subdivision génétique à l'échelle locale pour les trois espèces examinées; il y a une différentiation génétique faible, mais non-significative, entre les zones atlantique et cantabrique.

[Traduit par la Rédaction]

Introduction

The turbot (*Scophthalmus maximus*) is a marine flatfish of great commercial value. It is distributed all along European coasts, from Norway to Morocco in the Atlantic area, and across the Northern costs of Mediterranean up to the Black Sea (Blanquer et al. 1992). The decay of turbot fisheries in the last decade has been accompanied by an increase in its domestic production, one of the main problems in culture still being the low larval viability (which can reach values around 10% under optimal conditions). The existence of inbreeding depression in cultured populations has been suggested, considering their lower allozymic diversity than in natural populations and the negative correlation observed between heterozygosity and fluctuating asymmetry in bilateral meristic counts (Bouza et al. 1997). The reduced genetic

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diversity observed with allozymes in natural populations of turbot, much lower than other flatfish of the same genus sympatrically distributed along its natural range, has been explained by the existence of historical bottlenecks (Blanquer et al. 1992; Estoup et al. 1998). The lower gonadosomatic index and sperm volume of turbot compared with other flatfishes (Suquet et al. 1993; Lustres 1999) could also indicate the existence of genetic depletion in natural populations of this species (Bouza et al. 1997), an observation which should be taken with caution considering the different adaptive and evolutionary histories of the species analysed.

The application of highly variable markers in turbot has revealed a different picture than that of allozymes. For example, Coughland et al. (1998) reported similar levels of genetic diversity in natural populations and cultured strains of turbot by analysing three highly variable microsatellites. The study by Estoup et al. (1998), which focused on parentage assignment, did not evidence signal of genetic depletion for microsatellites in this flatfish species. These authors suggested that the different mutation rates between allozymes and microsatellites could account for their discordant genetic diversity, assuming the existence of historical bottlenecks in turbot. Different controls (conspecific populations or other related species that have not experienced a reduction in genetic diversity) have been considered to indirectly test for bottleneck hypotheses (O'Brien 1994). The use of closely related species appears to be the best available reference in turbot, because its low allozyme diversity has been reported over its entire geographic range (Blanquer et al. 1992).

Knowledge of population structure is of utmost importance for the use of fishery stocks and to evaluate the sources of genetic variation useful for culture enhancement. In contrast to inland fish species, the biogeography of flatfish communities in the northeastern Atlantic region has scarcely been addressed (Galleguillos and Ward 1982; Blanquer et al. 1992; Kotoulas et al. 1995). Previous analysis with allozymes had detected a very low population structuring in turbot, both along its natural range (Blanquer et al. 1992) and at a microgeographic scale (Bouza et al. 1997). One technical drawback of these studies was the very low genetic diversity of allozymes in turbot, which could hamper an appropriate estimation of population subdivision.

Genetic monitoring for species lacking allozyme variation has become more feasible since the application of hypervariable markers like microsatellites, which constitute genetic markers suitable to estimate evolutionary and population parameters (Estoup and Cornuet 1999). In this study, we have employed 12 microsatellite and 28 allozyme loci for the following reasons. (*i*) To analyse genetic diversity in natural populations of turbot in comparison with other flatfish species with similar geographic distribution: the brill (*Scophthalmus rhombus*) and the flounder (*Platichthys flesus*). These species show similar habitat and life-history features to turbot, including larval stage duration (Blanquer et al. 1992; Kotoulas et al. 1995). Our interest was to check if the low variation observed previously with allozymes in turbot could be extended to other genetic markers with higher mutation rates, and to test for evolutionary hypotheses which could explain a possible discordance between both set of markers. (*ii*) To check for losses of genetic diversity observed in culture due to mismanagement of broodstocks, previously reported using allozyme data. (*iii*) To determine whether flatfish populations of the Iberian Northeastern Atlantic fishery are panmictic or partially isolated population units.

Materials and methods

Sample collection

Two natural populations of each flatfish analysed (turbot, brill, and flounder) were collected in 1999 and 2000. Sampling focused on two areas putatively separated by a major oceanographic discontinuity (Harden Jones 1968): the Cantabric Sea and the Northeastern Atlantic Ocean in Galicia (northwest Spain). Two localities, approximately 350 km apart, were examined, namely Burela (43°40′N, 7°22′W) and Vilagarcía (42°36′N, 8°45′W). One sample of a commercial batch of turbot was also taken from one of the main European farms. All the samples consisted of about 50 individuals. The common name of each species (T, turbot; B, brill; F, flounder) and their origin (C, Cantabric; A, Atlantic; D, domestic) were used as population codes (i.e., TC represents natural turbot from the Cantabric Sea).

Microsatellite analysis

DNA was extracted from liver using the phenol–cloroform procedure. Polymerase chain reactions (PCR) were carried out using primers designed to amplify 12 previously characterized *S. maximus* microsatellites (Coughlan et al. 1996; Estoup et al. 1998; Table 1). Primers for loci Smax-01 to Smax-04 (Coughlan et al. 1996) were redesigned to optimize PCR amplifications. The original GenBank accession for locus Smax-04 (Z78099) contained another dinucleotide repeat ($(CA)_{23}$) upstream the 5′ primer binding site. We designed a new pair of primers to amplify this locus, termed as Smax-04b.

PCR were performed using 15 μL of mixture reaction containing 50 ng of template DNA, 100 μM of each dNTP, 15 pmol of each primer (the forward was fluorescently labelled with Cy5 (5-N-N′-diethyl-tetramethylindodicarbocyanine)), 1XPCR Gold Buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 0.5 units of Amplitaq Gold DNA polymerase (Applied Biosystems) and the $MgCl_2$ concentrations given in Table 1. PCR conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles at 94°C for 45 s, 50 s at the specific annealing temperature (Table 1), and 50 s at 72°C for extension. A final extension step was performed at 72°C for 10 min. The modifications applied for the amplification of brill and flounder microsatellites are presented in Table 1. Microsatellites were analysed on a Pharmacia ALFexpress II automatic sequencer (Amersham Biosciences Europe GmbH).

Allozyme analysis

Starch gel electrophoresis was performed on 18 enzymatic systems previously analysed in turbot (Bouza et al. 1997), with slight modifications when applied to brill and flounder. The relative electrophoretic mobility among species could be assessed at 21 loci out of the 31 assayed as follows (the most common allele of turbot was used as the reference to define the *100 allele): aspartate aminotransferase (s*AAT*-1*, s*AAT*-2*), alcohol dehydrogenase (*ADH**), adenylate kinase (*AK**), creatine kinase (*CK*-A1*, *CK*-C*), fumarase (*FH*-1*), glycerol-3-

Table 1. Set of primers and amplification conditions of the 12 microsatellite loci used in this study.

Name	Primer (5′→3′)	Core sequence	Annealing temperature, [MgCl$_2$](mM)		
			Turbot	Brill	Flounder
Smax-01	GGTGATAACGAGTTTGATGA TTCACAAGTAATCTCCCAAC	$(TG)_{20}$	52°C, 1.5	—	—
Smax-02	GGAGGATGTATTGAAAGTGT AGAGCAGGTCATTATACAGC	$(TG)_{16}$	56°C, 1.5	52°C, 1.3	52°C, 1.3
Smax-03	TGGTTAATTACAGCCTTTGG CTCATTTCCTTTTTGTTTGC	$(AC)_{16}$	50°C, 1.5	—	—
Smax-04	TCACAAAATCTGAACATCGGT GTAATCCACCAAACTGAACG	$(TG)_{20}$	55°C, 1.5	50°C, 1.8	—
Smax-04b	GGGTCATCATCTTTAGTCT GTCACAGTTTCATTAGGAG	$(AC)_{23}$	52°C, 1.3	52°C, 1.5	—
Sma3-8INRA	CCCTCCGTCAGACAAAGAG GACGAAGTTAATGTTTCATTG	$(GT)_{18}$–$(CT)_5$	52°C, 1.5	52°C, 1.3	—
Sma3-10INRA	CTATGCAAGTGAAGACACGTAC CTTTCACTCTGTTGTATCTAAGG	$(TG)_{50}$	55°C, 1.3	50°C, 1.3	—
Sma3-12INRA	CACAATTGAATCACGAGATG GCCACCACTGCGTAACAC	$(TG)_{21}$	55°C, 1.5	—	—
Sma4-14INRA	GCGAGCAAATATCAGAAGG CCAGAAACAGCTCCGACTC	$(GT)_{21}$	52°C, 1.2	54°C, 1.5	—
Sma5-111INRA	TCTACACTGCAGGTTGGG CTGATTATGGGCTGGACG	$(TG)_{13}$	52°C, 1.5	—	
Sma1-125INRA	CACACCTGACAAAGCTCAAC GCTGAACATTTTCATGTTGATAG	$(TAGA)_{11}$–$(TG)_4$	55°C, 1.3	52°C, 1.8	—
Sma3-129INRA	GCACTGCCTTTTCATTGG CAGCTCTAGATTGTTTATCCC	$(GT)_{29}$	55°C, 1.5	52°C, 1.3	—
Sma1-152INRA	GGGGAGGTGTCTGTCTGG GCACAGCTCCGAGCACAG	$(GT)_{14}$	52°C, 1.4	55°C, 1.5	—

phosphate dehydrogenase (*G3PDH*-1*, *G3PDH*-2*), isocitrate dehydrogenase (*IDHP*-1*, *IDHP*-2*), lactate dehydrogenase (*LDH*-A*), malate dehydrogenase (s*MDH*-A, s*MDH*-B*), malic enzyme (*MEP*-1*), phosphogluconate dehydrogenase (*PGDH*-1*), phosphoglucose isomerase (*GPI*-A*, *GPI*-B*), phosphoglucomutase (*PGM*-1*), sorbitol dehydrogenase (*SDH**), and superoxide dismutase (*SOD**). Interspecific electrophoretic mobility could not be assessed at the remaining 10 loci: esterase (*EST*-2*, *EST*-3*, *EST*-4*), lactate dehydrogenase (*LDH*-B*, *LDH*-C*), malic enzyme (*MEP*-1*), mannose-6-phosphate isomerase (*MPI*-1*, *MPI*-2*), phosphoglucomutase (*PGM*-2*), and xanthine dehydrogenase (*XDH**). Allele frequencies were estimated from zymograms at codominant loci. Allozyme data by Bouza et al. (1997) from Cantabric and Atlantic populations of turbot were included for genetic analyses. *MPI*-2* was excluded from analyses in the three species, together with EST and XDH systems in flounder, since they did not show adequate resolution. A final set of 28 loci in turbot and brill, and 24 loci in flounder, were included.

Data analysis

Unbiased expected heterozygosity (H_e), and mean number of alleles per locus (A) were computed to estimate genetic diversity (Nei 1987) at allozyme and microsatellite loci. Proportion of polymorphic loci (P_{99} and P_{95}) and variance of allele size (Var A; program RST-CALC 2.2; Goodman 1997) were additionally quantified to estimate genetic diversity at allozymes and microsatellites, respectively.

Departure from Hardy–Weinberg equilibrium (H–W) at all loci and genotypic linkage disequilibrium between pairs of loci were tested by exact test using GENEPOP 3.1 (Raymond and Rousset 1995). The sequential Bonferroni correction was employed when multiple tests were carried out (Rice 1989).

Homogeneity tests were performed between pairs of samples at allozyme and microsatellite loci by exact probability tests using GENEPOP 3.1. To test for genetic differentiation between samples, pairwise F_{ST} (FSTAT 2.9.1; Goudet 1995) and R_{ST} (RST-CALC 2.2) were estimated based on the infinite alleles model (IAM) and the stepwise mutation model (SMM), respectively. Their significance was checked by permutation tests. Genetic distances between samples were obtained from allozymes and microsatellites, by using two different estimators: D_A (Nei 1987; DISPAN program) and $(\delta\mu)^2$ (RST-CALC 2.2). Differences in genetic diversity and differentiation between species and markers were checked using Mann–Whitney U tests, and between populations within species using Wilcoxon's signed-ranks tests.

The family structure of the domestic strain (TD) was investigated by means of microsatellites. The existence of full-sib groups was analysed using PARTITION 1.0 (http://www.mscs.dal.ca/buttler/partition), following a likelihood procedure based on genetic similarity – genetic dissimilarity within and among groups (Herbinger–Smith algorithm). Concordance with the Mendelian laws was manually checked in the putative sib groups. The relatedness coefficient between individuals and groups was obtained using RELATEDNESS 5.2 (Queller and Goodnight 1989).

The demographic history of turbot and brill was explored using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) based on microsatellite data, under different mutational models: IAM, SMM and the intermediate two-phased model of mutation, TPM. This method is based on the faster reduction rate of the number of alleles than the expected heterozygosity (H_e; Nei 1987) after a population bottleneck (Nei et al. 1975). Significance of heterozygosity excess ($H_e > H_e$ at equilibrium) was checked using Wilcoxon's signed-ranks tests. The allele frequency distribution of microsatellites was also examined using BOTTLENECK 1.2.02, a mode shift from an L-shaped distribution being expected after a bottleneck.

Results

Microsatellites

Seven out of 12 microsatellite loci of turbot were satisfactorily resolved in brill, and only one locus was resolved in flounder (Table 1; Table A1). Natural samples of all species were in H–W equilibrium at all loci, since none of the 40 individual tests performed were found significant after sequential Bonferroni correction. By contrast, three out of the 12 loci analysed in the domestic strain of turbot, revealed significant departures from H–W equilibrium (Table 2). Exact probability tests for linkage disequilibrium between microsatellite loci rendered seven significant P values out of 66 pairs of loci tested (11%) after applying the sequential Bonferroni correction in the Cantabric population of turbot ($P = 0$; Smax-02/Sma5-111, Smax-02/ Sma3-12, Smax-04b/ Smax-04, Smax-04b/Sma3-8, Smax-04b/Sma4-14, Smax-04/ Sma4-14, Sma5-111/Sma4-14). The only significant test at the $\alpha = 5\%$ level observed in the Atlantic sample of turbot corresponded to the tightly linked Smax-04b/Smax-04 loci ($P = 0.006$). There was no evidence for linkage disequilibrium between any pairs of microsatellite loci studied in brill, their genotype proportions being largely concordant with those expected under gametic equilibrium.

Comparative values of genetic diversity per locus and per sample (Table 2), and between species (Table 3) showed no significant differences between natural populations of turbot and brill when comparing the common set of loci (H_e: 0.77 and 0.70, $P = 0.866$; A: 11.6 and 10.9, $P = 0.891$). Despite nonsignificance, the mean variance (Var A: 14.90 and 25.86, $P = 0.423$) and the mean range (36.02 ± 6.38 and 38.29 ± 9.99, $P = 0.436$) of allele size were lower in turbot than in brill. Significantly higher mean figures of H_e over 12 loci (H_e: 0.77 vs. 0.68, $P = 0.010$) and number of alleles (A: 11.25 vs. 6.50, $P = 0.002$) were observed in the natural populations than in the domestic strain of turbot analysed.

A strong family structure was observed in the domestic strain analysed (51 individuals). Four putative groups of full-sibs were detected (families I to IV with 11, 11, 6, and 5 individuals, respectively). These groups showed high average values of relatedness (R: 0.38 ± 0.06, 0.35 ± 0.08, 0.49 ± 0.08, and 0.43 ± 0.09) and agreement with Mendelian segregation at all loci. Family I could be additionally related to family III (R: 0.27 ± 0.09), suggesting a possible half-sib relationship. Many other minor groups with high relatedness coefficients were detected among the remaining individuals of the sample.

No evidence for significant heterozygosity excess was found for a recent population bottleneck either in turbot or in brill (Table 4). On the contrary: a significant heterozygosity deficiency was evidenced in turbot under the SMM and TPM, indicating too many alleles for the H_e observed, suggesting a population expansion for this species. The analysis of allele frequency distributions revealed typical L-shaped distributions in turbot and brill populations at all loci, which is expected when a population has not experienced a recent bottleneck.

There was no significant genetic differentiation between populations for all microsatellites analysed within turbot, brill, and flounder (12, 7, and 1 locus, respectively; Table 5). Similar figures of private alleles were detected in Atlantic and Cantabric populations of turbot, but a higher number of private alleles were detected at most loci studied in Atlantic than in Cantabric populations of brill.

Allozymes

Seventeen loci were polymorphic in at least one of the species analysed (Table A2), the remaining four being monomorphic (*FH**100 in turbot and brill, *FH**82 in flounder; *CK-C**100 in turbot and brill, *CK-C**98 in flounder; *SOD**100 and *AK**100 in the three species).

All samples were in H–W equilibrium at all codominant polymorphic loci analysed, since none of the 67 individual tests performed were found significant after sequential Bonferroni correction. There was no evidence of linkage disequilibrium between all pairs of allozyme loci tested in the three species studied.

Mean intraspecific estimates of genetic diversity were much lower in turbot (H_e 0.021; P_{95} 9%; P_{99} 20%) than in brill (H_e 0.093; P_{95} 33%; P_{99} 40%) and flounder (H_e 0.092; P_{95} 31%; P_{99} 48%; Table 3). The differences observed were significant with regard to brill ($P < 0.01$) and flounder ($P < 0.001$) with all estimators applied, and no statistical differences were detected between brill and flounder. On the other hand, the cultured strain of turbot, TD, showed lower genetic variability (H_e 0.006; P95 0%; P_{99} 16.7%; Table 3) than the natural samples of this species, the differences being marginally significant ($P = 0.051$) in H_e estimates, but not in the mean number of alleles ($P = 0.157$).

Genetic homogeneity was the rule between Cantabric and Atlantic populations within the three species studied (Table A2; Bouza et al. 1997). The average figures over loci within species were small and nonsignificant in all cases (Table 5). In agreement, low and nonsignificant absolute estimates of genetic differentiation were obtained in the three species turbot, brill, and flounder (D_A, Table 5). The main difference observed between both regions was the presence of private alleles. In brill and flounder, these alleles were mostly detected in the Atlantic populations (Table A2), which was in accordance with their higher genetic diversity values, especially the mean A and P_{99} (Table 3).

Discussion

Comparative genetic diversity analysis

In our study, positive amplifications of 12 microsatellite loci in turbot (Coughlan et al. 1996; Estoup et al. 1998) was obtained at seven loci in brill, and at only one locus in floun-

Table 2. Summary statistics for all microsatellite loci analysed in Cantabric (C) and Atlantic (A) natural samples of the three flatfish species studied, turbot, *Scophthalmus* maximus (T), brill, *Scophthalmus rhombus* (B), and flounder, *Platichthys flesus* (F), and in a cultured strain of turbot (TD).

Locus		Turbot			Brill		Flounder		Locus		Turbot			Brill	
		TC	TA	TD	BC	BA	FC	FA			TC	TA	TD	BC	BA
Smax-01	*A* (*ni*)	12(53)	10(46)	6(51)					Sma5-111	*A* (*ni*)	12(52)	13(46)	8(51)		
	A private	2	—	—					INRA	*A* private	1	2	—		
	A range	145–185	145–175	145–175						*A* range	256–286	250–278	256–276		
	A mean	166.8	163.9	166.7						*A* mean	267.7	265.7	264.0		
	Var *A*	12.32	14.66	4.66						Var *A*	7.67	9.33	7.22		
	H_e	0.752	0.765	0.645						H_e	0.890	0.900	0.807***		
Smax-02	*A* (n_i)	16(53)	18(46)	8(51)	15(48)	15(38)	4(16)	5(20)	Sma3-12	*A* (n_i)	12(52)	11(46)	8(51)		
	A private	1	3	—	2	3	—	1	INRA	*A* private	1	—	—		
	A range	93–137	93–141	95–137	93–131	85–131	81–91	81–91		*A* range	88–110	88–108	88–108		
	A mean	114.6	115.1	115.0	111.7	107.9	87.0	86.2		*A* mean	99.0	98.0	98.3		
	Var *A*	45.64	41.26	50.17	26.44	18.00	3.42	3.23		Var *A*	10.13	6.38	13.37		
	H_e	0.878	0.824	0.791***	0.812	0.752	0.621	0.695		H_e	0.825	0.819	0.795		
Smax-03	*A* (n_i)	7(53)	7(46)	5(51)					Sma4-14	*A* (n_i)	14(52)	10(46)	6(51)		
	A private	1	1	1					INRA	*A* private	4	—	—		
	A range	165–179	165–181	165–183						*A* range	116–158	116–138	122–138		
	A mean	172.4	173.3	173.4						*A* mean	135.3	128.6	128.7		
	Var *A*	8.38	10.29	9.21						Var *A*	14.31	3.68	2.76		
	H_e	0.605	0.638	0.615						H_e	0.788	0.796	0.639		
Smax-04b	*A* (n_i)	12(53)	12(45)	7(51)	11(48)	12(38)			Sma1-125	*A* (n_i)	7(53)	9(46)	7(51)	2(48)	3(38)
	A private	2	2	—	2	3			INRA	*A* private	—	1	—	—	1
	A range	88–128	88–134	88–128	100–154	98–142				*A* range	120–148	112–152	120–152	134–138	130–136
	A mean	112.3	115.0	112.3	127.6	120.7				*A* mean	132.6	132.4	136.6	136.0	134.0
	Var *A*	19.26	32.28	21.29	10.13	10.26				Var *A*	3.14	3.24	7.10	0.04	0.02
	H_e	0.694	0.769	0.726	0.846	0.815				H_e	0.730	0.756	0.805***	0.077	0.041
Smax-04	*A* (n_i)	5(53)	6(46)	3(51)	5(47)	8(38)			Sma3-129	*A* (n_i)	18(52)	16(46)	10(51)	20(48)	23(38)
	A private	—	1	—	—	3			INRA	*A* private	3	1	—	2	5
	A range	143–153	143–155	147–151	129–141	129–145				*A* range	157–199	163–215	157–189	137–219	137–213
	A mean	148.6	149.7	147.0	135.4	137.8				*A* mean	179.6	181.1	173.4	184.5	183.6
	Var *A*	0.55	0.91	0.40	1.18	1.55				Var *A*	19.65	21.81	20.99	115.49	152.92
	H_e	0.524	0.490	0.378	0.624	0.644				H_e	0.889	0.889	0.864	0.923	0.932
Sma3-8	*A* (n_i)	13(53)	13(46)	7(51)	11(46)	12(38)			Sma1-152	*A* (n_i)	9(53)	8(46)	3(51)	8(47)	8(38)
INRA	*A* private	—	—	—	3	4			INRA	*A* private	1	—	—	1	1
	A range	178–208	178–208	190–202	144–182	146–190				*A* range	91–109	91–107	95–99	93–107	93–109
	A mean	195.5	195.5	196.0	164.7	169.8				*A* mean	100.8	99.8	97.0	100.0	100.3
	Var *A*	8.25	6.81	3.22	9.99	10.16				Var *A*	2.57	3.14	0.28	3.12	2.75
	H_e	0.880	0.866	0.745	0.826	0.862				H_e	0.756	0.812	0.390	0.837	0.750

Note: For each locus and sample are included the number of individuals analysed (n_i), the number of alleles (*A*), the number of private alleles within species (*A* private), the range, mean, and variance of allele size (*A* range, *A* mean, Var *A*), the expected heterozygosity (H_e), and the significant departures from H–W expectations (***$P < 0.001$).

Table 3. Average figures of genetic diversity per sample over loci (n_l, number of loci) based on allozyme (*, data computed from Bouza et al. 1997) and microsatellite data.

Markers and estimates	Turbot TC	Turbot TA	Turbot TD	Brill BC	Brill BA	Flounder FC	Flounder FA
Allozymes							
n_l	28*	28*	24	28	28	24	24
Mean *A*	1.1	1.3	1.2	1.5	1.8	1.5	1.9
SE	0.1	0.1	0.1	0.1	0.2	0.2	0.3
Mean H_e	0.016	0.025	0.006	0.087	0.099	0.088	0.096
SE	0.009	0.011	0.002	0.030	0.030	0.032	0.030
$P_{95\%}$	10.7	7.1	—	30.0	36.7	25.8	36.9
$P_{99\%}$	10.7	28.6	16.7	33.3	46.7	40.5	55.3
Microsatellites							
n_l	12	12	12				
Mean *A*	11.4	11.1	6.5				
SE	1.1	1.0	0.6				
A private	16	11	1				
Mean var *A*	12.66	12.82	11.72				
SE	3.47	3.67	4.05				
Mean H_e	0.768	0.777	0.683				
SE	0.034	0.033	0.046				
n_l^a	7	7	7	7	7	1	1
Mean *A*	11.4	11.7	6.4	10.2	11.6		
SE	1.8	1.6	1.0	2.3	2.4		
A private	7	8	—	10	20	—	1
Mean var *A*	14.15	15.64	14.78	23.77	27.95		
SE	6.02	6.12	6.80	15.66	20.96		
Mean H_e	0.764	0.772	0.671	0.706	0.685		
SE	0.050	0.050	0.076	0.084	0.113		

Note: Two-letter species designations as in Table 2. Measures of genetic diversity: unbiased expected heterozygosity (H_e) and mean number of alleles (*A*) with their standard errors (SE); total number of private alleles (*A* private) within populations. The proportion of polymorphic loci (P_{95}, P_{99}: 95% and 99% criteria, respectively) and the variance of allele size, Var *A*, were included as estimates of genetic diversity for allozymes and microsatellites, respectively.

[a] In turbot, *Scophthalmus maximus*, the average estimates based on microsatellites were also calculated at the seven loci shared with brill, *Scophthalmus rhombus*, for interspecific comparison.

der, after the application of a wide set of experimental conditions. A similar result was obtained by Iyengar et al. (2000), who reported a cross-amplification of 12 and four loci in brill and *Pleuronectes platessa* (Pleuronectidae), respectively, starting from 18 turbot microsatellites. As expected, microsatellites loci in our study showed much higher and more significant genetic variation than allozymes in turbot and brill (only one microsatellite was analysed in flounder). However, sharp discrepancies were observed within markers between species, especially for allozyme loci. High genetic diversity was observed with allozymes in brill and flounder populations, as reported for most Pleuronectiformes with respect to the average values in marine fishes (Galleguillos and Ward 1982; Blanquer et al. 1992; Kotoulas et al. 1995). This is in contrast to the low figures exhibited by turbot (Blanquer et al. 1992; Bouza et al. 1997). This lower allozymic variability in turbot was confirmed with all estimators applied after the analysis of a large sample of loci, and no significant differences were observed between brill and flounder. Conversely, microsatellites did not show significant differences in genetic diversity between natural populations of turbot and brill. This intermarker discrepancy has been argued on the existence of historical bottlenecks along turbot evolution (Blanquer et al. 1992; Bouza et al. 1997; Estoup et al. 1998), considering that the number of generations for recovering genetic diversity is inverse to mutation rate (Lande and Barrowclough 1987). Under this bottleneck hypothesis turbot microsatellites would have reached the mutation – genetic drift equilibrium, while protein loci would not have.

However, a more detailed analysis of microsatellite data in turbot and brill suggests that lower diversity values than expected also occur in turbot. Most microsatellites amplified in our work were selected in turbot attending to the highest polymorphic criterion for parentage assignment (Estoup et al. 1998); therefore, a bias towards higher diversity values should be expected in this species. By contrast, these loci would probably represent a random sample of loci in brill. The much higher variance among loci in brill than in turbot for all genetic diversity estimators supports this assertion. Interspecific comparisons of genetic diversity with microsatellites have usually detected a higher polymorphism in the

Table 4. Departures from the null hypothesis that all loci fit (under different mutational models: IAM, infinite alleles; SMM, stepwise mutation; TPM, two-phased model of mutation) mutation–drift equilibrium (BOTTLENECK 1.2; Cornuet and Luikart 1996) in Cantabric and Atlantic populations of turbot and brill (TC, TA and BC, BA, respectively).

	IAM			SSM			TPM		
Sample[a]	n_l ex.	P ex.		n_l ex.	P ex.		n_l ex.	P ex.	
TC (12)	8	0.065	No deviations	1	0.999	Deficiency**	3	0.999	Deficiency**
TA (12)	9	0.055	No deviations	2	0.999	Deficiency**	2	0.999	Deficiency**
BC (7)	5	0.234	No deviations	1	0.992	Deficiency*	1	0.980	No deviations
BA (7)	5	0.531	No deviations	0	1.000	Deficiency**	0	1.000	Deficiency**

Note: The number of loci showing heterozygosity excess (n_l ex.) was indicated. P values are for Wilcoxon's signed-ranks (one-tailed) tests for heterozygosity excess (P ex.), and significance of the departures from equilibrium (two-tailed tests) adjusted after sequential Bonferroni correction for each species and model of mutation ($^*P < 0.05$; $^{**}P < 0.01$).

[a] The total number of loci studied is in parentheses.

Table 5. Estimates of genetic differentiation between populations (C, Cantabric; A, Atlantic; D, turbot cultured strain) within species (T, turbot; B, brill; F, flounder) based on allozymes and microsatellites.

	Allozymes			Microsatellites				
Samples	n_l	F_{ST}	D_A	n_l	F_{ST}	R_{ST}	D_A	$(\delta\mu)^2$
TC–TA	28	0.002^{NS}	0.0043^{NS}	12	0.002^{NS}	0.008^{NS}	0.0408^{NS}	0.5317^{NS}
BC–BA	28	0.025^{NS}	0.0106^{NS}	7	0.005^{NS}	0.003^{NS}	0.0463^{NS}	0.5130^{NS}
FC–FA	24	0.001^{NS}	0.0127^{NS}	1	-0.008^{NS}	-0.006^{NS}	—	—

Note: The number of loci (n_l) used for each species and marker is included. NS, nonsignificant.

focal species than in the related ones (Jarne and Lagoda 1996). In spite of this, in our study there were no significant differences in genetic diversity between turbot and brill.

Exact probability tests evidenced significant linkage disequilibrium between two tightly linked microsatellite loci (Smax-04b/Smax-04) in natural populations of turbot, whereas there was no such evidence in brill, natural samples of both species being in H–W equilibrium at all loci analysed. Several other disequilibriums appeared in the Cantabric sample of turbot, but not in the Atlantic one, which excludes an explanation based on a general bottleneck in the whole species. Nearly complete linkage exists among Smax-04b and Smax-04 loci in turbot since they are no more than a few base pairs apart. The same linkage probably also occurs in brill, taking into account the small DNA segment considered and the close evolutionary relationships between species. If a bottleneck occurred along turbot evolution, the physical linkage between both loci would probably preclude the recovering of gametic equilibrium, as it occurs in this species but not in brill, since there is a very low recombination rate between these loci (Hartl and Clark 1997). This result, although limited to a single couple of tightly linked loci, also points to the existence of bottlenecks along turbot evolution.

The impact of putative historical bottlenecks on turbot heterozygosity was assessed using the BOTTLENECK method by Cornuet and Luikart (1996), based on microsatellite data. Neither significant heterozygosity excess nor deviations from the expected allele frequency distribution were observed. These findings do not support for a recent population bottleneck in this species. Conversely, according to SMM and TPM, a heterozygosity deficiency occurs in turbot and brill. This suggests a population expansion in the recent past of these two species, probably related to the recolonisation process of the northern areas during the last postglacial time.

In summary, although evidence suggests historical bottlenecks in turbot, the information obtained from the BOTTLENECK method indicates that this event did not occur very recently. The low mutation rate of allozymes precludes an accurate estimation of the time of occurrence, assuming an inverse relationship between mutation rate and the recovering of genetic diversity from the putative bottleneck (Lande and Barrowclough 1987).

A main question arises from these results: What are the effects of the postulated bottleneck (inbreeding depression and genetic depletion) on fitness traits in culture, or even in natural populations of turbot? Some reports have shown that a loss of genetic variation, even in a few generations, can be associated with fitness decrease (Saccheri et al. 1998). Quantitative genetics theory predicts a purification of deleterious alleles after a bottleneck; therefore, the effects of depression being more important in a recent bottleneck (Hedrick 2001). However, slightly deleterious alleles could be fixed after bottlenecks determining moderate genetic load in natural populations for longer periods (Hedrick 2001). As a result, some sperm parameters (volume, gonadosomatic index, and sperm abnormalities), used as indicators of genetic depletion in endangered species (O'Brien 1994), have revealed poorer figures in turbot than in other flatfish, including brill and flounder (Suquet et al. 1993; Lustres 1999). Nevertheless, these observations should be interpreted with caution since differences in life-history traits might also reflect different adaptative responses among species. On the other hand, despite the low variability observed for allozymes in our study, substantial genetic variation was found for the highly variable microsatellite loci. Moreover, the study of fluctuating asymmetry in cultured turbot suggests the presence of genetic variation for fitness traits (Bouza et al. 1997). These results indicate that substantial genetic variability for microsatellites and fitness traits has been recovered from the putative bottleneck, probably as a result of their high mutation rates. All these data, though not conclusive, lead to a recommendation for careful broodstock foundation in turbot and its appropriate management in culture.

Genetic diversity in turbot culture

The cultured strain of turbot, TD, showed lower allozyme heterozygosity than the natural samples of this species. Similar findings have been reported in Bouza et al. (1997). Hypervariable microsatellite loci also confirmed the loss of genetic variation, specifically by a sharp decreasing in the mean number of alleles, as expected according to theoretical predictions (Nei et al. 1975). A main question, however, is how representative of the farmed broodstocks are the domestic samples analysed (Bouza et al. 1997; this study). A strong family structure could be observed in the sample TD, suggesting very few breeders involved in obtaining these commercial batches. The ongoing analysis of the broodstocks of one of the main turbot farms in northwest Spain has evidenced much higher genetic diversity values than in TD, which is similar to natural populations (our unpublished data). A similar outcome was observed in other European farmed strains of turbot (Coughlan et al. 1998; Estoup et al. 1998). These results clearly indicate that the commercial batches, like TD, are not representative with regard to the broodstocks in turbot culture, and suggest caution against the use of these populations for broodstocks foundation in turbot.

Intraspecific and interspecific genetic differentiation

Studies on genetic structure of flatfish in the northeastern Atlantic region have focused on a few species, mainly based on allozymes (Galleguillos and Ward 1982; Blanquer et al. 1992; Kotoulas et al. 1995). As in other marine fish species, little geographic divergence has been observed (Ward et al. 1994). A strong intraspecific genetic homogeneity was observed in our study between Cantabric and Atlantic populations of turbot and brill analysed with both sets of markers. As in flounder, with allozymes, no significant differentiation was detected with relative or absolute estimators of genetic divergence. The unique exception to this intraspecific homogeneity was the presence of private alleles, mostly rare, and mainly from the Atlantic populations, at both sets of markers. The null differentiation found at microsatellites for turbot and brill, and at allozymes for the three flatfish studied suggests high migration rates in these species.

A good correlation between the pattern of microsatellite diversity previously reported (Coughlan et al. 1996; Estoup et al. 1998) and our own data could be observed. The range of allele size and frequency distribution at seven common microsatellite loci in the North Cantabric population found by Estoup et al. (1998) showed a great concordance with our results. The main discrepancy was related to the allelic nomenclature used in both studies, a slippage of the allelic series by one or two bp being observed at some loci: Sma1-125INRA, Sma3-8INRA, Sma3-12INRA, Sma4-4INRA, Sma3-129INRA, and Sma1-152INRA. Because the primers for loci Smax-01 to Smax-03 (Coughlan et al. 1996) were redesigned in our study, the allelic series and ranges appeared accordingly modified by a constant related to the new location, but showed very similar absolute ranges and frequency distributions to the samples from Norway and South Ireland analysed by these authors. Assuming correspondence in the allelic nomenclature, North Atlantic turbot samples (Coughlan et al. 1998; Estoup et al. 1998) showed great genetic similarity to northwest Spain populations. The genetic distance and relative differentiation calculated at the seven common loci between the North and South Cantabric samples analysed by Estoup et al. (1998) and ourselves, respectively, were very low (mean D_A: 0.0660; G_{ST}: 0.008; program DISPAN). This suggests high gene flow in turbot, even between geographically distant populations, a similar picture to that previously revealed at a macrogeographical scale by allozyme markers (Blanquer et al. 1992).

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Appendix A

Table A1. Allele frequencies at 12 previously characterized turbot microsatellites (Coughlan et al. 1996; Estoup et al. 1998) assayed in the three flatfish species analysed in the present study: 12, seven, and one out of 12 loci resulted positively amplified in turbot (T), brill (B), and flounder (F), respectively.

Locus	Allele	TC (30)	TA (19)	TD (28)	BC (25)	BA (44)	Locus	Allele	TC (30)	TA (19)	TD (28)	BC (25)	BA (44)
Smax-01	145	0.075	0.109	0.010	—	—	Sma5–111	250	—	0.011	—	—	—
	152	0.009	0.011	—	—	—	INRA	256	0.058	0.141	0.088	—	—
	159	0.019	0.011	—	—	—		258	0.135	0.141	0.176	—	—
	163	0.019	0.022	—	—	—		260	0.154	0.152	0.294	—	—
	165	0.028	0.076	—	—	—		262	0.115	0.141	0.108	—	—
	167	0.387	0.326	0.520	—	—		264	0.144	0.087	0.039	—	—
	169	0.292	0.337	0.029	—	—		266	0.154	0.098	0.020	—	—
	171	0.104	0.076	0.088	—	—		268	0.019	0.022	—	—	—
	173	0.038	0.011	0.078	—	—		270	0.087	0.043	0.245	—	—
	175	0.009	0.022	0.275	—	—		272	0.087	0.076	—	—	—
	177	0.009	—	—	—	—		274	0.019	0.054	—	—	—
	185	0.009	—	—	—	—		276	0.019	0.011	0.029	—	—
								278	—	0.022	—	—	—
Smax-02	81	—	—	—	—	—		286	0.010	—	—	—	—
	83	—	—	—	—	—							
	85	—	—	—	—	0.010	Sma3-12	88	0.288	0.283	0.108	—	—
	87	—	—	—	—	—	INRA	90	0.058	0.054	—	—	—
	89	—	—	—	—	—		92	0.269	0.261	0.167	—	—
	91	—	—	—	—	—		94	0.087	0.163	0.010	—	—
	93	0.019	0.033	—	0.237	0.146		96	0.038	0.033	0.020	—	—
	95	0.292	0.391	0.304	0.053	0.031		98	0.048	0.087	0.020	—	—
	97	0.019	0.033	—	0.342	0.458		100	0.038	0.022	—	—	—
	99	0.038	0.054	0.147	0.132	0.125		102	0.010	0.033	—	—	—
	101	0.038	0.043	—	0.026	0.031		104	0.029	0.011	0.127	—	—
	103	—	—	—	0.039	0.063		106	0.096	0.043	0.324	—	—
	105	—	—	—	0.013	0.010		108	0.029	0.011	0.225	—	—
	107	0.104	0.033	0.265	0.013	—		110	0.010	—	—	—	—
	109	0.094	0.098	0.010	—	—							
	111	0.028	0.011	—	—	—	Sma4-14	116	0.058	0.011	—	—	—
	113	—	0.011	0.108	0.026	0.021	INRA	122	0.029	0.033	0.010	—	—
	115	—	—	—	0.013	0.010		124	0.048	0.076	0.029	—	—
	117	0.075	0.054	—	—	0.010		126	0.144	0.217	0.353	—	—
	119	0.028	0.033	—	0.039	0.031		128	0.096	0.065	—	—	—
	121	0.085	0.076	—	0.026	0.021		130	0.096	0.174	0.098	—	—
	125	0.047	0.043	0.010	—	0.010		132	0.413	0.348	0.480	—	—
	127	—	0.022	—	0.013	—		134	0.029	0.033	—	—	—
	129	0.047	0.033	—	—	—		136	0.010	0.011	—	—	—
	131	—	0.011	—	0.013	0.021		138	0.019	0.033	0.029	—	—
	133	0.019	—	—	—	—		140	0.010	—	—	—	—
	135	0.038	—	0.020	—	—		154	0.010	—	—	—	—

Table A1 (*concluded*).

Locus	Allele	TC (30)	TA (19)	TD (28)	BC (25)	BA (44)
	137	0.028	0.011	0.137	—	—
	141	—	0.011	—	—	—
	149	—	—	—	0.013	—
Smax-03	165	0.594	0.522	0.559	—	—
	167	0.038	0.054	0.167	—	—
	171	0.019	0.011	—	—	—
	173	0.104	0.076	0.029	—	—
	175	0.009	—	—	—	—
	177	0.057	0.033	—	—	—
	179	0.179	0.293	0.225	—	—
	181	—	0.011	—	—	—
	183	—	—	0.020	—	—
Smax-04b	88	0.009	0.011	0.020	—	—
	90	0.066	0.122	0.069	—	—
	92	0.019	0.011	—	—	—
	98	—	—	—	—	0.010
	100	—	—	—	0.013	0.010
	108	—	—	—	—	0.042
	110	0.019	—	—	—	—
	114	0.009	0.044	—	—	—
	116	0.198	0.156	0.167	—	—
	118	0.509	0.433	0.127	0.026	0.010
	120	0.075	0.067	0.461	0.118	0.083
	122	0.019	0.011	—	0.039	0.042
	124	0.009	—	—	0.184	0.281
	126	0.028	0.033	0.137	0.224	0.198
	128	0.038	0.056	0.020	0.079	0.031
	130	—	—	—	0.237	0.250
	132	—	0.033	—	0.053	0.031
	134	—	0.022	—	—	—
	142	—	—	—	—	0.010
	150	—	—	—	0.013	—
	154	—	—	—	0.013	—
Smax-04	129	—	—	—	0.039	0.011
	133	—	—	—	0.421	0.457
	135	—	—	—	0.447	0.383
	137	—	—	—	—	0.043
	139	—	—	—	0.066	0.064
	141	—	—	—	0.026	0.011
	143	0.009	0.054	—	—	0.021
	156	0.010	—	—	—	—
	158	0.029	—	—	—	—
Sma1-125 INRA	112	—	0.011	—	—	—
	120	0.264	0.185	0.294	—	—
	124	0.028	0.011	—	—	—
	128	0.075	0.109	0.167	—	—
	130	—	—	—	—	0.010
	132	0.208	0.228	0.176	—	—
	134	—	—	—	0.961	0.979
	136	0.396	0.391	0.186	—	—
	138	—	—	—	0.039	0.010
	140	0.009	0.033	0.010	—	—
	148	0.019	0.022	0.020	—	—
	152	—	0.011	0.147	—	—
Sma3-129 INRA	137	—	—	—	0.081	0.144
	151	—	—	—	—	0.011
	153	—	—	—	0.054	0.022
	155	—	—	—	0.095	0.100
	157	0.010	—	0.029	0.041	—
	163	0.029	0.011	0.108	0.054	0.033
	165	0.038	—	—	0.203	0.111
	167	—	0.011	0.069	0.095	0.078
	169	0.221	0.185	0.147	—	—
	171	0.192	0.196	0.049	—	—
	173	0.067	0.109	0.059	0.041	0.011
	175	0.106	0.054	—	0.014	0.033
	177	0.048	0.033	0.216	—	—
	179	0.019	0.011	—	—	0.011
	181	0.010	0.022	—	—	—
	183	0.038	0.022	0.118	—	0.022
	185	0.019	0.054	0.010	—	—
	187	0.029	0.065	—	—	—
	189	0.058	0.141	0.196	—	0.011
	191	0.077	0.054	—	—	—
	193	0.019	0.022	—	0.054	0.011
	195	0.010	—	—	0.027	0.033
	197	—	—	—	0.027	0.011
	199	0.010	—	—	0.095	0.122
	201	—	—	—	0.027	0.056
	203	—	—	—	0.014	0.044
	205	—	—	—	0.014	0.044

	145	—	—	—	—	0.011
	147	0.642	0.696	0.775	—	—
	149	0.245	0.141	0.137	—	—
	151	0.094	0.087	0.088	—	—
	153	0.009	0.011	—	—	—
	155	—	0.011	—	—	—
Sma3-8 INRA	144	—	—	—	0.014	—
	146	—	—	—	0.042	0.022
	158	—	—	—	0.042	0.054
	162	—	—	—	0.097	0.174
	164	—	—	—	0.111	0.120
	166	—	—	—	0.278	0.163
	168	—	—	—	0.278	0.207
	170	—	—	—	0.069	0.174
	172	—	—	—	—	0.022
	174	—	—	—	0.028	—
	176	—	—	—	—	0.011
	178	0.009	0.011	—	0.028	—
	182	—	—	—	0.014	0.011
	184	—	—	—	—	0.033
	186	0.066	0.054	—	—	—
	188	0.009	0.011	—	—	—
	190	0.038	0.065	0.098	—	0.011
	192	0.123	0.098	0.029	—	—
	194	0.113	0.283	0.441	—	—
	196	0.038	0.065	0.029	—	—
	198	0.160	0.109	0.167	—	—
	200	0.094	0.141	0.108	—	—
	202	0.226	0.076	0.127	—	—
	204	0.085	0.065	—	—	—
	206	0.019	0.011	—	—	—
	208	0.019	0.011	—	—	—
	207	—	—	—	—	0.011
	209	—	—	—	0.027	0.022
	211	—	—	—	0.014	0.044
	213	—	—	—	0.014	0.011
	215	—	0.011	—	—	—
	219	—	—	—	0.014	—
Sma1-152 INRA	91	0.038	0.043	—	—	—
	93	—	—	—	0.013	0.011
	95	0.066	0.076	0.039	0.079	0.053
	97	0.302	0.283	0.206	0.237	0.415
	99	0.377	0.272	0.755	0.211	0.074
	101	0.057	0.065	—	0.079	0.106
	103	0.066	0.130	—	0.197	0.245
	105	0.075	0.120	—	0.145	0.085
	107	0.009	0.011	—	0.039	—
	109	0.009	—	—	—	0.011

Note: Gene frequencies correspond to Cantabric and Atlantic populations of turbot and brill (TC, TA and BC, BA, respectively) and to the cultured strain of turbot (TD). The data at the only locus amplified in flounder (Smax-02) is not presented and its summary statistics were included in Table 2. Loci are sorted in numerical order from top to bottom and from left to right. The number of individuals analysed in each sample is in parentheses.

Table A2. Allelic frequencies at the 21 polymorphic isozyme loci detected in four natural samples from northwest Spain of brill (B) and flounder (F), and a cultured strain of turbot (TD).

Locus	Allele	TD (30)	BC (19)	BA (28)	FC (25)	FA (44)
sAAT-1*	100	1.000	1.000	1.000	1.000	0.986
	110	—	—	—	—	0.014
sAAT-2*	51	—	—	—	—	0.095
	56	—	0.053	0.037	—	—
	70	—	—	0.037	—	—
	80	—	—	—	1.000	0.905
	100	1.000	0.842	0.833	—	—
	140	—	0.105	0.093	—	—
ADH*	86	—	0.263	0.250	—	—
	100	1.000	—	—	—	—
	143	—	0.711	0.679	—	—
	167	—	—	—	0.917	1.000
	210	—	0.026	0.071	—	—
	267	—	—	—	0.083	—
CK-A*	100	1.000	1.000	1.000	1.000	0.942
	120	—	—	—	—	0.058
EST-4*	76	—	—	—	NR	0.020
	87	—	—	—		0.040
	91	—	—	—		0.040
	96	—	—	—		0.420
	100	1.000	1.000	1.000		0.480
G3PDH-1*	60	—	0.342	0.411	—	—
	77	0.017	—	—	—	—
	100	0.983	0.658	0.571	—	—
	112	—	—	—	0.021	0.012
	125	—	—	—	0.666	0.670
	137	—	—	0.018	—	—
	140	—	—	—	0.292	0.295
	163	—	—	—	0.021	0.023
G3PDH-2*	100	1.000	1.000	0.923	—	—
	115	—	—	0.077	—	—
	136	—	—	—	1.000	1.000
GPI-A*	100	1.000	0.947	0.982	—	—
	160	—	—	—	1.000	1.000
	260	—	0.053	0.018	—	—
LDH-A*	21	—	—	—	0.958	1.000
	35	—	—	—	0.042	—
	60	—	1.000	1.000	—	—
	100	1.000	—	—	—	—
sMDH-1*	70	—	—	0.056	—	—
	82	—	—	—	1.000	0.989
	100	1.000	1.000	0.944	—	—
	139	—	—	—	—	0.011
sMDH-2*	44	—	—	—	0.958	1.000
	49	—	—	—	0.021	—
	59	—	—	—	0.021	—
	90	—	0.026	0.071	—	—
	100	1.000	0.948	0.911	—	—
	107	—	0.026	0.018	—	—
MEP-1*	58	—	0.029	0.037	—	—
	62	—	—	—	0.025	0.013
	70	—	—	—	—	0.013
	73	—	0.765	0.630	—	—
	78	—	—	—	0.600	0.685
	80	—	0.206	0.333	—	—
	98	—	—	—	0.375	0.289
	100	0.983	—	—	—	—
	110	0.017	—	—	—	—
MPI-1*	100	1.000	1.000	1.000	—	—
	140	—	—	—	1.000	0.977
	165	—	—	—	—	0.023
MPI-2*	100	0.900	0.895	0.840	—	—
	106	—	—	—	0.095	0.011
	110	0.100	—	—	—	—
	112	—	0.105	0.160	—	—
	120	—	—	—	—	0.114
	133	—	—	—	0.905	0.875
PGDH-1*	17	—	—	—	1.000	0.977
	23	—	—	—	—	0.023
	75	0.017	—	—	—	—
	100	0.983	1.000	1.000	—	—

GPI-B*	80	—	1.000	1.000	—	—	*PGM*-1*	68	—	—	—	0.229	0.193
	93	—	—	—	0.042	0.057		82	—	—	0.054	—	—
	95	—	—	—	0.958	0.943		85	—	—	—	0.771	0.807
	100	0.983	—	—	—	—		97	0.017	—	—	—	—
	105	0.017	—	—	—	—		100	0.983	1.000	0.928	—	—
								120	—	—	0.018	—	—
IDHP-1*	45	—	—	0.018	—	—							
	100	1.000	1.000	0.946	—	—							
	180	—	—	0.036	—	—	*SDH**	28	—	—	—	—	0.023
	186	—	—	—	0.917	0.954		30	—	—	—	0.955	0.884
	242	—	—	—	0.083	0.023		33	—	—	—	—	0.035
	298	—	—	—	—	0.023		36	—	—	—	0.045	0.058
								49	—	—	0.018	—	—
IDHP-2*	57	—	—	—	0.021	—		62	—	0.079	0.071	—	—
	85	—	—	0.018	—	—		82	—	0.921	0.911	—	—
	95	—	—	—	0.937	0.910		100	1.000	—	—	—	—
	100	1.000	0.974	0.982	—	—							
	105	—	—	—	0.021	0.045	*XDH**	76	—	—	0.018	NR	NR
	110	—	0.026	—	—	—		96	—	0.316	0.018		
	114	—	—	—	0.021	0.045		100	1.000	0.684	0.964		

Note: Natural populations: Cantabric (C) and Atlantic (A). The commonest allele of turbot was used as the reference to define the *100 allele. Allozyme data from Cantabric and Atlantic populations of turbot included in genetic analyses were taken from Bouza et al. (1997). Loci are sorted in numerical order from top to bottom and from left to right. NR, not resolved; The number of individuals analysed in each sample is in parentheses.

Least median of squares: a suitable objective function for stock assessment models?

Kyle W. Shertzer and Michael H. Prager

Abstract: Robust fitting methods, intended for data sets possibly contaminated with invalid observations, are gaining increased use in analysis of fishery data. In particular, the method of least median of squares (LMS) has attracted attention. Its hallmark is high statistical resistance, which makes it immune to up to 50% contamination in the data. However, the same property makes it inefficient and can cause faulty fitting of typical fishery data. The LMS fit can be in conflict with important sections of a time series, a problem we illustrate by fitting a biomass dynamic (surplus production) model to simulated and actual fishery data. Additionally, we illustrate that LMS parameter estimates can be highly sensitive to small perturbations in the data. Other robust methods, like the method of least absolute values (LAV), appear less prone to such problems. A key reference on LMS recommends using the method as part of an exploratory procedure to identify outliers, rather than as an objective function for final model fitting. We concur with that recommendation.

Résumé : Les méthodes d'ajustement robustes destinées aux ensembles de données potentiellement contaminées par des observations invalides sont de plus en plus utilisées dans l'analyse des statistiques de pêche. En particulier, la méthode des moindres médianes des erreurs au carré (LMS) a suscité beaucoup d'intérêt. Sa caractéristique principale est sa forte résistance statistique qui lui permet de supporter une contamination représentant jusqu'à 50 % des données. Cependant, cette même caractéristique la rend inefficace et peut occasionner un mauvais ajustement de données du type généralement obtenu dans les pêches. L'ajustement de LMS peut être en désaccord avec de longues sections des séries temporelles; c'est un problème que nous illustrons en ajustant un modèle dynamique de biomasse (de production excédentaire) à des données simulées et réelles de pêche. De plus, nous démontrons que les estimations des paramètres de LMS peuvent être très sensibles à de petites perturbations dans les données. D'autres méthodes robustes, telles que la méthode des moindres valeurs absolues (LAV), semblent moins sujettes à ces problèmes. Un travail essentiel sur les LMS recommande d'ailleurs d'utiliser la méthode comme une des procédures exploratoires pour identifier les données aberrantes, plutôt que comme fonction objective pour l'ajustement final du modèle. C'est là une recommandation que nous entérinons.

[Traduit par la Rédaction]

Introduction

Most fishery models are fit, in whole or in part, by least-squares (LS) estimation. Some models explicitly use LS as the objective function; others use maximum likelihood or conditional maximum likelihood, which in many cases are equivalent to LS. Least squares has an extensive literature, and its estimates have many attractive features; however, a widely recognized drawback of LS is its sensitivity to outlying values (Rousseeuw and Leroy 1987). Thus, in a relatively small data set, typical in fishery work, a single outlying observation that results in a large residual (or a few such observations) can have relatively strong influence (in the ordinary sense) on the resulting estimates. In many cases, one would prefer that the most outlying points not have such great influence, and it is for such cases that robust fitting methods have been devised.

When errors satisfy the Gauss–Markov assumptions of independence and identical $N(0,\sigma^2)$ distribution, LS is optimal in linear regression in the sense of providing minimum variance, unbiased estimates (Casella and Berger 1990). Because of its desirable properties in linear fitting, LS is widely used in nonlinear fitting, as well. However, real data sets often violate the Gauss–Markov assumptions, and that can have substantial effects on the LS parameter estimates. In particular, data used in stock assessments are likely to violate those assumptions because of large measurement and process errors (Hilborn and Walters 1992) that may not be normally distributed, even under transformation. Poorly defined error structure and the widespread existence of outliers in fishery data sets make robust methods attractive for fitting stock assessment models (Chen and Jackson 1995, 2000).

There are two approaches to using robust methods to reduce the impact of outliers (Rousseeuw and Leroy 1987). The first approach identifies outliers based on the residuals from a robust model fit. Once identified, outliers are corrected, removed, or downweighted; then the "good" data are refit using standard LS methods. This approach was illustrated in fishery analyses by Chen and Paloheimo (1994), Restrepo and Powers (1997), and Prager (2002), among others.

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The second approach to using robust methods simply replaces the LS objective function with one less sensitive to outliers, i.e., a robust objective function. That approach was illustrated by Chen and Andrew (1998), who proposed use of least median of squares (LMS) as a "potentially useful addition to methods used to fit production models to abundance index and catch data". That paper has been influential: in a recent working group, we observed analysts using LMS as the objective function for production models, citing Chen and Andrew (1998). We found that alarming, because problems can arise with such uses of LMS. The goals of this paper are to illustrate those problems and to discourage the use of LMS as a replacement for standard methods of model fitting.

LMS

The method of LMS is a well-known robust fitting method. As with any regression, the goal of LMS is to estimate p model parameters, $\boldsymbol{\theta} = (\theta_1,\ldots,\theta_p)$, from the data. A LMS estimate $\hat{\boldsymbol{\theta}}$ is one that minimizes the objective function

$$\text{(1)} \qquad \text{median } r_i^2, \qquad i = 1,2,\ldots,n$$

where the residuals, r_i, are the differences between observed and predicted responses.

LMS is devised to have a high breakdown point, usually defined as the smallest percentage of "contaminated" data needed to shift the estimate by an arbitrary amount (Rousseeuw and Leroy 1987). In linear regression, the LMS breakdown point is 50%; the corresponding LS breakdown point is zero (Rousseeuw 1984).

Other robust methods

Ordinary least squares falls under the more general category of M estimators (Rousseeuw and Leroy 1987; Berk 1990). Such estimators are based on minimizing an objective function of the form

$$\text{(2)} \qquad \sum_{i=1}^{n} \rho(r_i)$$

where ρ is a symmetric function with a unique minimum at zero (Rousseeuw and Leroy 1987). For example, $\rho(x) = x^2$ defines the LS objective function. Other functions can be used that reduce the influence of outliers and are thus considered more robust than LS. Such functions generally increase at a slower rate than a quadratic, either for all x or for all x greater than some value. The method of LAV (Harter 1985), a robust M estimator used here, is defined by $\rho(x) = |x|$. Other M estimators worth mentioning, but not receiving full attention here, are the Huber, bi-square, and Bell M estimators (Berk 1990). The Huber estimator uses the LS objective function up to some predetermined residual value, and beyond that uses the LAV objective function; the bi-square estimator uses a function that initially increases quickly (like Huber), but is constant after some residual value; the Bell estimator is similar to bi-square, but with a smoother function that approaches a constant as its limit.

LMS is considered to be an S estimator, which minimizes a type of robust M estimate of scale on the residuals (for detailed description of S estimators, see Rousseeuw and Leroy (1987)). Least trimmed squares (LTS), another widely used S estimator, is LS performed after removing a user-defined fraction of the highest squared residuals (Rousseeuw 1984). Both LMS and LTS attempt to minimize some robust measure of scatter in the residuals.

Nonlinear analyses

In general, stock-assessment models are nonlinear. Robust and resistant objective functions, although initially developed (like least squares) for linear models, can also be applied (like least squares) to nonlinear cases. Unfortunately, minimization of the objective function (i.e., parameter estimation) is usually much more difficult in nonlinear analyses, particularly when the objective function is not smooth (Gill et al. 1981). LMS, being an extreme case of a nonsmooth function, poses a particularly difficult challenge in nonlinear optimization (Stromberg 1993). In general, the properties of LMS in nonlinear analyses have not been as thoroughly studied as in linear analyses. In nonlinear analyses, LMS is considered a high-breakdown estimator, but definition of the breakdown point varies and breakdown analysis becomes more complex (Stromberg and Ruppert 1992). Nonetheless, LMS has been found useful for detecting outliers in nonlinear regression (Stromberg 1993).

Robust methods in fishery management

Robust methods have been applied to several problems in fishery science; we mention only some applications here. Chen and Paloheimo (1994) compared the relative merits of LS, LMS, LMS-based reweighted LS, and LAV for estimating mortality rates and the catchability coefficient from catch and effort data. Prager et al. (1995) used robust regression in fitting length-conversion equations for billfish. Stock–recruitment relationships have been analyzed with a variety of robust methods, using both simulated data and real data on several species (Chen and Paloheimo 1995; Wang and Liu 1999). Robust methods have been used in several studies to fit population dynamic models to time-series data. Such analyses have examined both surplus production models without age structure (Chen and Andrew 1998; Chen and Montgomery 1999; Prager 2002) and assessment models of catch at age (Restrepo and Powers 1997).

Trouble with LMS

In this section, we present four examples that demonstrate inherent difficulties possible when using LMS as an objective function. The first two examples are based on simulated data; the last two are based on real data.

Our first example demonstrates instability of LMS in a linear regression problem. However, the potential for instability in LMS is not limited to linear regression. High-breakdown methods like LMS achieve robustness by essentially ignoring outlying data, and such data may be signal, not noise. Thus, the high-breakdown property can lead to poor fits of distinct sections of data, especially when fitting autocorrelated time-series data.

The remaining examples illustrate potential pitfalls when using LMS to fit a logistic (Schaefer 1954, 1957) surplus production model (here chosen to represent a nonlinear fishery model) to simulated and real data sets on catch and relative abundance. The production model formulation, detailed by Prager (1994) and reviewed by Quinn and Deriso (1999), postulates that the rate of biomass change follows the differential equation

$$(3) \qquad \frac{dB_t}{dt} = (r - F_t)B_t - \frac{r}{K}B_t^2$$

Here, B_t is the population biomass at time t, F_t is the fishing mortality rate at time t, r is the population's intrinsic growth rate, and K is the carrying capacity. Typically, F is converted from fishing effort rate f_t assuming constant catchability q so that $F_t = qf_t$. Integrating eq. 3 projects biomass over time and further integration (of F_tB_t) projects the corresponding yields Y_t.

Given observations on effort rates and yields, one can estimate model parameters r, K, q, and B_0. Estimates from surplus production models can be used to derive management benchmarks, particularly maximum sustainable yield (MSY) and fishing effort at MSY (f_{MSY}). Under the assumption of logistic population growth, $\widehat{\text{MSY}} = \hat{r}\hat{K}/4$ and $\widehat{f_{\text{MSY}}} = \hat{r}/(2\hat{q})$.

The method of parameter estimation is described in detail by Prager (1994) and was implemented here with the computer program ASPIC (Prager 1995). The fitting method is an observation-error estimator conditioned on observed yield. The estimator was originated in this context by Pella (1967) and was termed the "time-series" method by Hilborn and Walters (1992). Objective functions (LS, LAV, LMS) were computed from residuals in the abundance index in logarithmic transform. In nonlinear LMS analysis, it can be extremely difficult to locate the global minimum of the objective function. The software used requires repeated restarts of its algorithm to the same point to accept a solution, even provisionally. For this study, the fitting software was modified to repeatedly refit over a grid of initial guesses, and the best solution so obtained was adopted.

In examining the results from the three objective functions, we compare predicted and observed relative abundances. We also compare the estimates of four quantities of management interest: MSY, f_{MSY}, F in the last estimated year relative to F at MSY($F./F_{\text{MSY}}$), and stock biomass in the last estimated year relative to the stock biomass at MSY ($B./B_{\text{MSY}}$).

Example 1: instability in linear regression

In linear regression, LMS is robust to 50% contamination of the data, providing a high breakdown point. However, this does not mean, as one might expect, that LMS estimates are insensitive to small perturbations in the data. To the contrary, LMS estimates can be quite unstable (Hettmansperger and Sheather 1992). Although they are insensitive to outliers, LMS estimates can jump between solution regimes in response to small changes in the data, as may occur when adding new observations or with shifts in existing observations.

We demonstrate the potential for LMS instability in a widely familiar setting: linear regression. We generated 31 pairs of (x,y) data according to

$$(4a) \qquad x_i = i, \quad i = 0,\ldots,30$$

$$(4b) \qquad y_i = \begin{cases} 0, & i = 0,1,\ldots,14 \\ 1, & i = 15 \\ 2, & i = 16,17,\ldots,30 \end{cases}$$

We then fit the model $Y = \theta_1 + \theta_2 X$ by estimating θ_1 and θ_2 under three different objective functions: LS, LAV, and LMS. On this data set, the three solutions are quite similar (Fig. 1*a*). An interesting characteristic of LMS is that solutions are not always unique, and here, two distinct LMS solutions exist (Fig. 1*a*).

Two small changes in the data set illustrate the instability of LMS. When the centrally located point (x_{15}, y_{15}) is increased from (15, 1) to (15, 2), the LS and LAV lines hardly change, but the LMS line changes markedly (Fig. 1*b*), from $y = -0.21 + 0.08x$ (or $y = -0.29 + 0.08x$) to $y = 2$, a horizontal line! When, instead, the central point is decreased from (15, 1) to (15, 0), the LS and LAV fits again change very little, but the LMS fit jumps to $y = 0$, a second horizontal line (Fig. 1*c*). This example demonstrates that LMS estimates can be much more sensitive to small data changes than LS and LAV estimates (Figs. 1*a*–1*c*).

By design, the LMS objective function essentially ignores almost half the data. Thus, shifts in which points contribute to the objective function can occur abruptly with small changes in a single data value, leading to marked changes in parameter estimates (Fig. 1*d*). In general, instability of LMS estimates can occur whenever the data can be divided into two partial samples with approximately equal residual values, such that small perturbations can cause the LMS estimate to shift from fitting one partial sample to fitting the other (Hettmansperger and Sheather 1992).

Example 2: instability on simulated fishery data

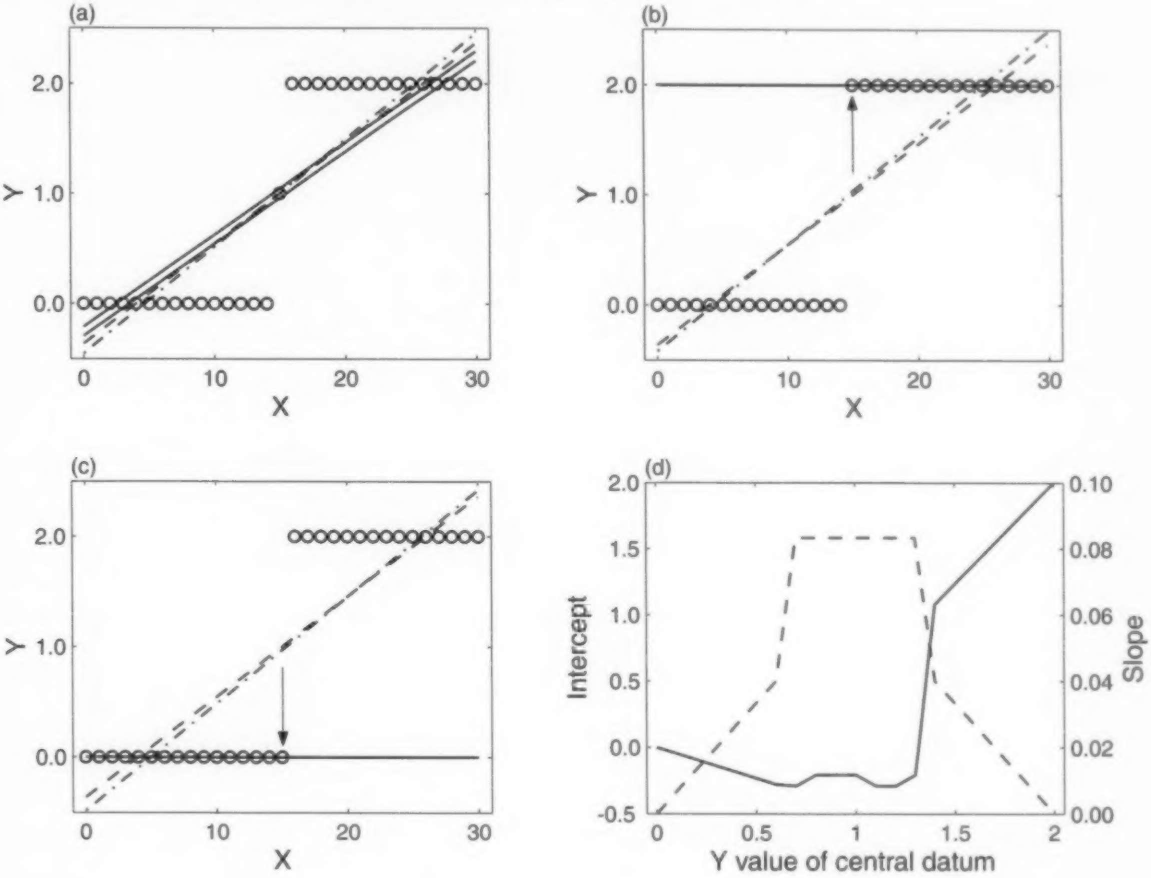
The next example uses a simulated fishery data set to demonstrate the potential instability of LMS in fishery modeling. We generated time series of fishing effort, biomass, and yield, assuming that stock dynamics follow eq. 3 with $r = 1$, $K = 1000$, $q = 1$, and initial biomass $B_0 = 0.5K$. The applied F relative to F_{MSY} first remains constant at a relatively low level, then increases, and then decreases sharply, as under a sudden, strong management regime (Fig. 2*a*). The resulting trajectory of biomass declines in response to the increased F, and then rebounds (Fig. 2*b*). The simulation provided a noise-free time series of yield, to which we added simulated lognormal observation errors

$$(5) \qquad \tilde{Y}_t = Y_t \exp(v_t), \quad v_t \sim N(0,\sigma^2)$$

We simulated observed yields $\tilde{Y}_t$ using $\sigma = 0.1$, and thus generated a 19-year catch–effort data set. Using the LS, LAV, and LMS objective functions, we then fit the surplus production model in three ways: (*i*) to the first 15 years of simulated data, (*ii*) to the first 17 years of simulated data, and (*iii*) to the entire 19 years of simulated data. This is analogous to repetition of a stock assessment when additional data become available.

On the 15-year time series, the LS and LAV fits are very similar (Fig. 3*a*), and produce similar estimates of management benchmarks. However, the LMS fit is strikingly different, reproducing eight of the data points quite well and seemingly ignoring the other seven (Fig. 3*a*). The LMS estimate of MSY is comparable to those of LS and LAV, but LMS estimates of the remaining management quantities differ considerably (Table 1). With two years of data added to the time series, the LS and LAV fits change little, whereas the

Fig. 1. Examples of linear regression ($Y = \theta_1 + \theta_2 X$) using three different objective functions: least squares (· –), least absolute values (– –), and least median of squares (LMS) (—). LMS can be nonunique and in (*a*) gives two solutions. Data (○) are (*a*) original data (see text), (*b*) central datum shifted up, (*c*) central datum shifted down. (*d*) Sensitivity of LMS estimates, intercept $\hat{\theta}_1$ (—) and slope $\hat{\theta}_2$ (– –), to changes in the *Y* value of only the central datum; remaining data as in Figs. 1*a*–1*c*.



LMS fit jumps to a new solution regime (Fig. 3*b*). This jump is reflected in the estimated management quantities (Table 1). With an additional two years added to form a 19-year time series, the LS and LAV fits still remain largely unchanged, but the LMS fit jumps yet again (Fig. 3*c*). As before, shifts in the LMS-estimated management quantities accompany the change in fit (Table 1). In this example, each time the simulated stock assessment is repeated as additional data become available, estimates from the LMS objective function provide a very different picture of stock dynamics and management implications.

Examples using actual fishery data

The preceding examples used artificial data constructed to illustrate troubles with LMS. We now demonstrate that similar fitting problems can occur on real fishery data sets. As did Chen and Andrew (1998), we fit logistic surplus production models to time series of fishery data. Our examples use data on Atlantic menhaden (*Brevoortia tyrannus*) and barramundi (*Lates calcarifer*). The following analyses are used for illustration and are not intended to define status of these stocks. Although data were obtained from reputable sources, they may include preliminary or unpublished values, and no attempt has been made to verify or update the data sets, nor to compare results to those of other models that might be applicable.

Example 3: Atlantic menhaden

Data on Atlantic menhaden are those used in Vaughan et al. (2002), and consist of total landings in weight and estimates of *F* (on a weight basis), derived from virtual population analysis, for age 2^+ fish. Those derived estimates of *F* were used instead of recorded data on fishing effort because of the density-dependent catchability demonstrated in this species (Schaaf and Huntsman 1972).

When the menhaden data are fit by LS, the fitted line of relative abundance attempts to capture the high relative abundances near the start of the time series (Fig. 4*a*). Because those relative abundances are somewhat inconsistent with simple production model dynamics (Vaughan et al.

Fig. 2. Simulated data used to illustrate fitting problems with least median of squares: (*a*) trajectory of relative fishing mortality rate; (*b*) resulting trajectory of relative biomass.

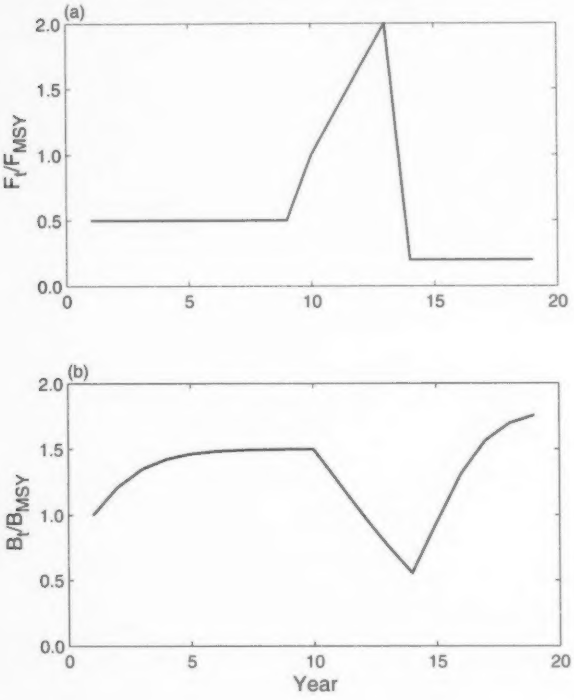
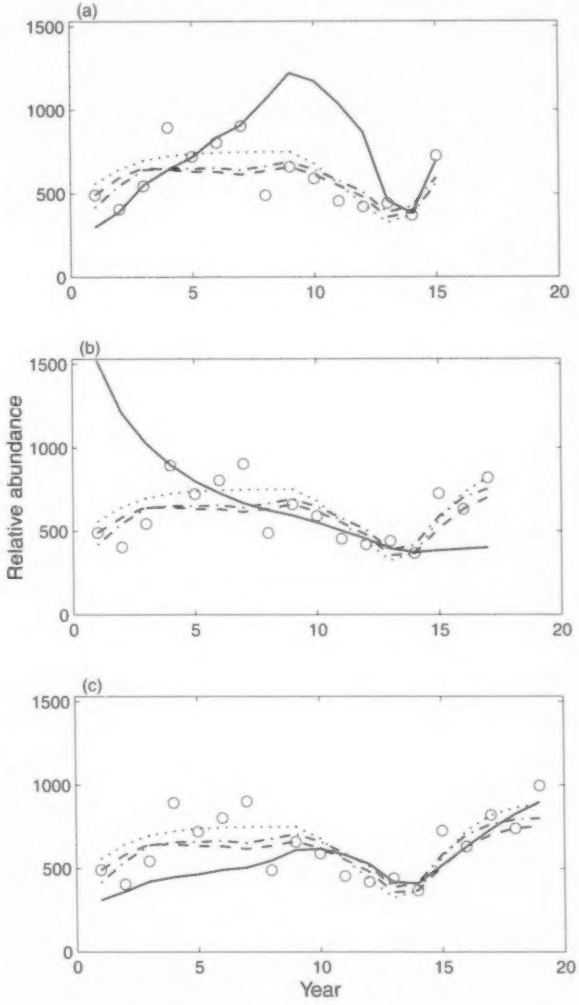


Fig. 3. Production model fits to simulated abundance index data (○) using three objective functions: least squares (· –), least absolute values (– –), and least median of squares (—). "True" underlying simulated values also shown (···). (*a*) Fits based on the first 15 years of data, (*b*) fits based on the first 17 years, and (*c*) fits based on the full data set (19 years).



2002), the fit cannot capture the stock dynamics faithfully. The LAV fit is similar to the LS fit in that the estimated dynamics approximate the observed data (Fig. 4*b*). However, the LMS fit, which aims only at the lowest median residual, essentially ignores the early high abundances, and in those years it predicts much lower abundances than were observed (Fig. 4*c*). The period of high abundance in the 1950s is well documented in the menhaden literature (e.g., Schaaf and Huntsman 1972; Ahrenholz et al. 1987; Vaughan et al. 2002) and is quite unlikely to be an error; thus, the LMS fit conflicts with an important observed phenomenon.

Estimates of management-related quantities from LS and LAV are similar (Table 2). The estimate of sustainable yield (MSY) from LMS is similar to estimates from the other objective functions, but LMS estimates of other management quantities are quite different. The LMS fit describes a stock that supplies the same MSY at higher F_{MSY}, which by necessity would be applied to a smaller B_{MSY} (Table 2).

Example 4: barramundi

Data on barramundi, 1973–1989, were extracted from table 4.1 of King (1995). Data are missing for 1976; our fitting method accommodates the missing value of relative abundance, and we assumed that the 1976 catch was equal to the mean of the 1975 and 1977 catches.

Fits from LS and LAV display similar patterns, differing mostly in the first year (Figs. 5*a*, 5*b*). The LMS fit is less convincing, and resembles the fit to the Atlantic menhaden data in that the initial period of high abundance is not fit at all (Fig. 5*c*). Given the difference in fit, one might expect to see large differences in management estimates from the different objective functions. However, that does not occur in this case (Table 2). It appears that stock dynamics in the latter portion of the data set are sufficiently consistent with the earlier portion that failure to fit the early portion (as in the LMS fit of Fig. 5*c*) does not seriously affect the results.

Discussion

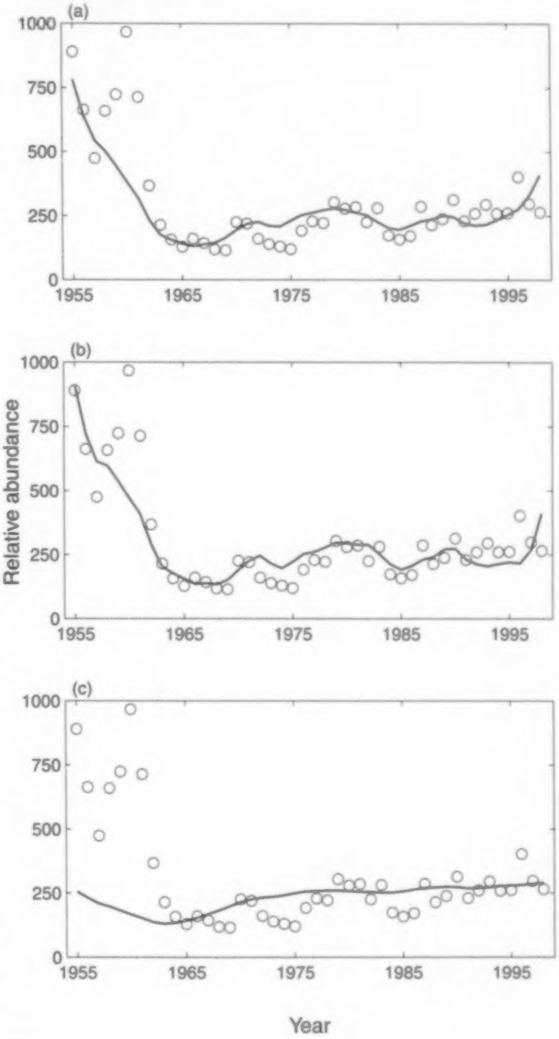
The goal of this study was to illustrate potential problems with use of LMS as an objective function, particularly when fitting time-series data. (We did not attempt to compare

Table 1. Comparison of estimated quantities from application of logistic surplus production model to simulated data under three objective functions and with three sample sizes *N*.

		Estimates			
Objective function	N	MSY	f_{MSY}	$F_{\cdot}/F_{\mathrm{MSY}}$	$B_{\cdot}/B_{\mathrm{MSY}}$
True values	15	250	0.50	0.20	0.94
True values	17	250	0.50	0.20	1.56
True values	19	250	0.50	0.20	1.76
LS	15	271	0.64	0.19	1.54
LS	17	268	0.62	0.17	1.78
LS	19	261	0.59	0.21	1.80
LAV	15	266	0.65	0.20	1.50
LAV	17	251	0.59	0.20	1.71
LAV	19	250	0.59	0.23	1.77
LMS	15	251	0.29	0.36	1.00
LMS	17	125	0.42	0.49	1.36
LMS	19	244	0.40	0.28	1.52

Note: "True" underlying simulated values also shown. Quantities estimated: MSY, maximum sustainable yield; f_{MSY}, fishing effort rate at MSY; $F_{\cdot}/F_{\mathrm{MSY}}$, ratio of final-year fishing mortality rate to that at MSY; $B_{\cdot}/B_{\mathrm{MSY}}$, ratio of final-year population biomass to that at which MSY can be obtained. LS, nonlinear least squares; LAV, least absolute values; LMS, least median of squares.

Fig. 4. Production model fits (—) to observed abundance index data (○) on Atlantic menhaden (*Brevoortia tyrannus*) using three objective functions: (*a*) least squares, (*b*) least absolute values, and (*c*) least median of squares.



LMS to other objective functions as to bias or variance of estimates: to do so would require extensive simulation studies.) We have encountered problems with LMS in our own work, and given the increasing interest in robust methods in general and LMS in particular, we believe that it is important for fishery biologists to be aware of potential shortcomings. A recurring pattern is that the LMS objective function can ignore portions of the data in searching for the lowest median residual. Such portions can be distinct sections of a time series that carry signals of biological importance. This is implicit in the definition of LMS, but it may not be appropriate for analysis of fishery time series.

At first glance, the problem of ignoring contiguous observations may seem of no importance when fitting non-time-series data (e.g., length–weight data). However, the same issue can arise whenever data cluster into distinct groups that can be described by different parameter values. When more than 50% of the data fall into one such group, the LMS solution can reflect only that group. When the data form two distinct groups, there will be two candidate LMS solutions, potentially with very different parameter estimates, and the choice between them will depend on the relative sample sizes and dispersions in each group. Small data changes (e.g., adding an observation) can move the solution from describing one group to the other, as illustrated in our examples and discussed by Hettmansperger and Sheather (1992) and Ellis (1998). Any data set, even a noisy one, in which roughly half the data define one set of parameter estimates and the other half define a second set, would be subject to instability as data are added or small changes made to central values.

The desired property of LMS is its resistance to outliers. However, resistance comes at a cost: loss of efficiency (i.e., slower convergence of the estimator to its true value with increasing sample size). Stromberg (1993) summarizes research on this topic, including a proof that the asymptotic efficiency of LMS in linear regression is zero. That finding is supplemented by simulations showing that, in linear regression, LMS is quite low in efficiency compared to LS on finite samples with normal errors (Rousseeuw and Leroy 1987). The tradeoff between resistance and efficiency is evident in other robust methods, and in general, higher breakdown is accompanied by lower efficiency (Berk 1990). In general, such issues have been poorly studied in nonlinear applications, in part because of the large universe of nonlinear models.

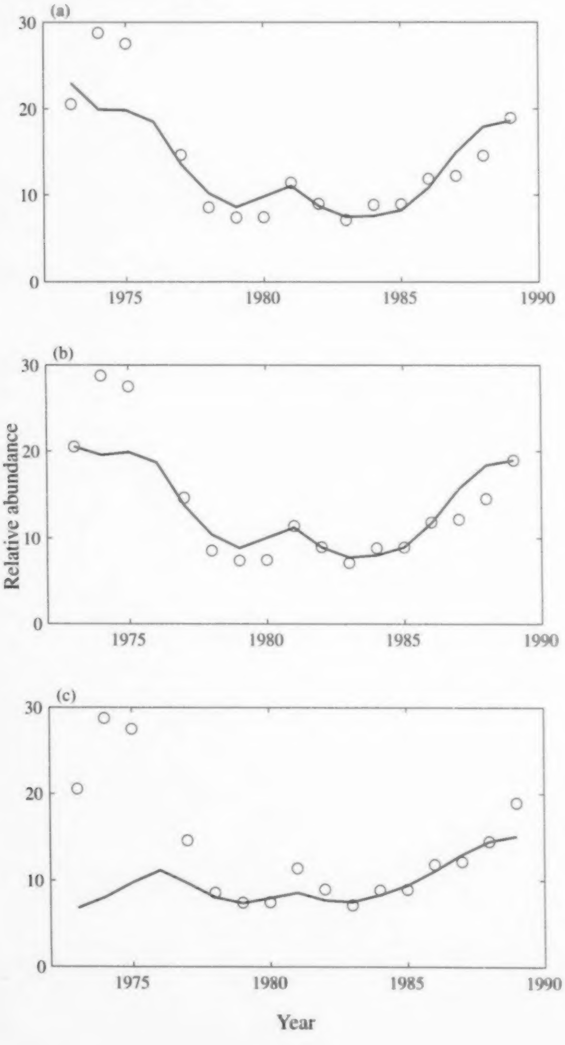
A practical problem with the use of LMS is the difficulty of finding the minimum of a LMS objective function, partic-

Table 2. Comparison of estimated quantities from application of logistic surplus production model to fishery data sets under three objective functions.

Species	Objective function	MSY	f_{MSY}	$F./F_{MSY}$	$B./B_{MSY}$
Atlantic menhaden	LS	461	0.95	0.63	0.94
Atlantic menhaden	LAV	530	0.86	0.69	0.83
Atlantic menhaden	LMS	402	2.04	0.42	1.48
Barramundi	LS	750	59.1	0.54	1.46
Barramundi	LAV	747	58.0	0.54	1.47
Barramundi	LMS	758	72.3	0.54	1.45

Note: Quantities estimated: MSY, maximum sustainable yield; f_{MSY}, fishing effort rate at MSY; $F./F_{MSY}$, ratio of final-year fishing mortality rate to that at MSY; $B./B_{MSY}$, ratio of final-year population biomass to that at which MSY can be obtained. These examples are not intended to define the status of any stock. LS, nonlinear least squares; LAV, least absolute values; LMS, least median of squares.

Fig. 5. Production model fits (–) to observed abundance index data (○) on barramundi (*Lates calcarifer*) using three objective functions: (*a*) least squares, (*b*) least absolute values, and (*c*) least median of squares.



ularly on nonlinear data (as here). This difficulty, which is recognized in the statistical literature (Stromberg 1993), has at least two components. First, the LMS objective function is not differentiable, nor is it continuous, a situation that precludes the use of gradient optimization methods and makes locating a global minimum much more difficult for other methods (Gill et al. 1981). Second, the LMS solution to a particular problem can be nonunique (as in our first example), and even when the solution is formally unique, it may be nonunique in practical terms. By that, we mean that many LMS minima may exist, all with extremely small residuals. In production modeling, we have found data sets with numerous local minima in which the median residual was less than 0.01% of the actual abundance-index value. As such minima can correspond to markedly different sets of parameter values, we consider such solutions nonunique in practical terms. Differences in the LMS objective function among such local minima can be small enough to result from numerical issues such as roundoff error (in data entry or computation) or choice of convergence criteria.

Because of the troubles associated with using LMS for fitting data, we recommend that it be used in detection of outliers, rather than as a substitute for the usual least-squares objective function in fishery population models. Outlier identification is the use advised by Rousseeuw and Leroy (1987), a standard statistical treatise on robust and resistant linear methods. It has been advocated elsewhere in the fishery literature (Chen and Paloheimo 1994; Chen et al. 1994; Chen and Jackson 2000), though not always adhered to in practice (Chen and Montgomery 1999). By placing LMS in a diagnostic role, the analyst will be in a position to examine LMS results and determine how they might be useful in each particular case.

LMS offers two main advantages in outlier detection. First, outliers cannot always be detected simply by plotting the data, particularly in multivariate data. In such situations, LMS provides an objective method for diagnosis. Second, outliers may exist in independent variables as well as (or instead of) in dependent variables. Such outliers, called "leverage points", can have relatively small residuals, making outlier identification based on these residuals misleading. At least in linear analyses, LMS is robust to outliers in both dependent and independent variables (Rousseeuw and Leroy 1987).

Once identified, outliers in fishery data can be corrected, removed, downweighted, or perhaps included based on background information related to the investigation (Chen and Jackson 1995; Restrepo and Powers 1997; Prager 2002).

Then, the possibly modified data set can be fit by some more efficient method, like LS (Rousseeuw and Leroy 1987). An alternative might be to fit all the data using a robust method that does not suffer the problems outlined for LMS. For this, we suggest that investigators consider the method of LAV. That method has the advantages of using all the data and yet being more robust to outliers than LS. In addition, it is relatively simple to implement and similar enough to LS that it can be explained easily. A potential drawback of LAV is that it is not robust to leverage points. However, in the context of fitting time-series data, where the independent variable is the year of observation, it seems unlikely that leverage points would exist.

In conclusion, naive use of the method of LMS can be dangerous, particularly when fitting time series or other autocorrelated data. This danger applies to other robust methods in proportion to their statistical resistance. The method of LAV, though robust, does not share LMS's high resistance, and it may thus be more desirable. In all cases, it seems preferable that outlier downweighting and subsequent fitting be done through a process that involves human thought, and not be relegated to an automatic procedure using a robust or resistant method. The unquestioned value of such methods is in detecting outliers. The decision to change or discount the data is best left to the subject-matter expert.

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Algal production and trihalomethane formation potential: an experimental assessment and inter-river comparison

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Abstract: Trihalomethanes (THMs) are byproducts produced during the disinfection of drinking water. We combined survey and experimental approaches to identify factors that influence THM formation potential (THMFP) in the Ohio River drainage basin. Two surveys of the Ohio River and its five principal tributaries were conducted to characterize spatial variation in THMFP in relation to algal abundance and suspended organic matter. We performed three experiments by placing Ohio River water in 2000-L outdoor mesocosms and manipulating algal senescence and bloom development by shading. Increases in THMFP among high- and low-light and dark tanks suggest that algal production, algal senescence, and possibly photolysis increased THMFP by as much as 50% over 3–6 days. Comparable yields of THMs (per unit of chlorophyll) were observed in both survey and experimental settings. Comparison of input waters with outputs indicates that the Ohio River at times acts to attenuate downstream transport of THM precursors. Our findings suggest that both watershed-scale and internal processes regulating THMFP should be considered as utilities develop strategies to meet new drinking water guidelines.

Résumé : Les trihalométhanes (THMs) sont des produits secondaires générés durant la désinfection de l'eau potable. La combinaison d'inventaires et d'expériences nous a permis d'identifier les facteurs qui influencent le potentiel de formation de THM (THMFP) dans le bassin hydrographique de l'Ohio. Deux inventaires de l'Ohio et de ses cinq principaux tributaires ont servi à caractériser la variation spatiale du THMFP en fonction de l'abondance des algues et de la matière organique en suspension. Nos trois expériences consistaient à placer de l'eau de l'Ohio dans des mésocosmes extérieurs de 2000 L et à manipuler la sénescence et la prolifération des algues au moyen de l'ombrage. L'augmentation du THMFP dans les mésocosmes exposés à la lumière forte ou faible, ou gardés à l'obscurité, semble indiquer que la production d'algues, la sénescence des algues et peut-être aussi la photolyse augmentent le THMFP d'une valeur pouvant atteindre 50 % sur une période de 3–6 jours. Des quantités comparables de THM (par unité de chlorophylle) ont été observées dans les inventaires et dans les expériences. La comparaison des eaux à l'arrivée et à la sortie montre que l'Ohio peut quelquefois atténuer le transport vers l'aval des précurseurs des THM. Nos résultats indiquent que, lorsque les services publics mettront au point des stratégies pour se conformer aux nouvelles normes régissant l'eau potable, ils devront tenir compte tant des processus qui agissent à l'échelle du bassin hydrographique que de ceux qui fonctionnent au niveau interne dans la régulation du THMFP.

[Traduit par la Rédaction]

Introduction

Chlorination is a common practice worldwide used to ensure the safety of water for drinking supplies. Although this procedure is effective in ensuring potable water, the process of chlorination may lead to the formation of disinfection byproducts (DBPs). These include trihalomethanes (THMs) such as chloroform and bromoform (Rook 1976) that have been shown to be carcinogenic in laboratory animals (Singer 1999). New United States Environmental Protection Agency (USEPA) regulations require water utilities to take steps to reduce regulated DBPs by 2004 (Cooke and Kennedy 2001). As a result, there is an increased urgency to understand how source waters differ in THM formation potential (THMFP) and what role ecological processes play in regulating the production and fate of THM precursors. THM levels in raw water are usually very low (Arguello et al.1979), and therefore utilities have focused on engineering solutions to reduce THM formation during treatment. Although effective, such proximate solutions do not address the important question of why source waters vary widely in THMFP. If the environmental factors favoring high THMFP can be better understood, this would provide utilities with options for managing source waters to meet the new guidelines. Various site-specific studies have correlated THMFP with environmental factors such as temperature, pH, and concentrations of particulate organic carbon (POC) and dissolved organic carbon (DOC) or their constituent components (e.g., humic acids; Singer 1999; Lin et al. 2000). Although these studies have broadly characterized the environmental conditions favoring THM formation, there has been much less emphasis placed

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on understanding the processes which give rise to THM precursors. Lakes, rivers, and reservoirs are commonly used as source waters and THM precursors may originate from autochthonous or allochthonous inputs of DOC and POC. Potential autochthonous sources include sediments, macrophytes, and algae (Palmstrom et al. 1988). Algae are of particular interest because they attain high numbers in some water supplies and produce extracellular products (ECPs) that may serve as THM precursors (Hoehn et al. 1980). In a combined reservoir and experimental study, Graham et al. (1998) found a positive correlation between THM and chlorophyll concentrations. They also assessed THMFP in algal cultures and found that THM yield was highest in the exponential and death stages of the cultures and was lowest in the stationary phase.

Allochthonous sources of THM precursors include terrestrially derived organic materials that have undergone varying degrees of diagenesis. External sources of THM precursors may be particularly important in rivers and reservoirs because these systems receive higher allochthonous inputs of organic matter than lentic waters. In addition, their higher loads of suspended particulate material reduce light penetration and may limit internal generation of THM precursors associated with phytoplankton production (Alpine and Cloern 1992; Cole et al. 1992). Stepczuk et al. (1998*a*, 1998*b*, 1998*c*) used estimates of organic carbon loading and other parameters to model temporal and spatial variability of THM precursors in a river–reservoir system in New York state (U.S.A.). Landscape level approaches have potential for identifying important sources and sinks at the basin level and for developing whole-catchment strategies for mitigating THM formation.

Although rivers are widely used as sources of drinking water, we know of no prior process-oriented studies on THMFP in rivers. The Ohio River basin drains 10% of the continental United States and includes a population of over 25 million people. More than three million users depend directly on the Ohio River as a water supply. The tributaries contributing to the Ohio River drainage network differ in their degree of hydrologic regulation, watershed land use, and water quality, all of which may influence THMFP. In this study, we combine survey and experimental approaches to examine the relationship between THMFP, algal production, and DOC and POC. We tested the hypothesis that algal growth enhanced THMFP by (*i*) comparing rivers that differed in algal production, and (*ii*) experimentally manipulating algal production in mesocosms containing riverine phytoplankton communities. We predicted that differences in THMFP among river basins could be explained in part by variation in algal abundance (chlorophyll) and that experimental inducement of algal blooms in mesocosms would result in increases of THMFP.

Materials and methods

Comparative studies

Our assessment of spatial covariation in THMFP, chlorophyll (chl *a*), and organic carbon (POC and DOC) focused on the lower Ohio River and its major tributaries (Kentucky, Green, Wabash, Tennessee, and Cumberland rivers; Fig. 1). These tributaries represent all inflows within the study reach that contribute at least 1% of the volume of the Ohio River at its confluence with the Mississippi. Surveys were conducted during the warmwater, low-flow period in August and October of 2000. Sampling followed a semi-Lagrangian design with upstream sites visited 3–7 days prior to downstream sites depending on transit time (estimated from river stage). The mainstem Ohio River was represented by an upstream site located near Vevay, Ind., at Ohio River kilometre (ORK) 854, a midstream site near Evansville, Ind. (ORK 1251) and a downstream site near the confluence with the Mississippi River (ORK 1501). Tributary samples were collected a minimum of 1 km above their confluence with the Ohio River to ensure that the sample was representative of water originating within the sub-basin. Water and ancillary field data (temperature, pH, turbidity) were taken at a midpoint in the river channel and within 1 m of the surface. A single water sample was collected at each site and analyzed for THMFP, chl *a*, POC, and DOC. Flux rates were derived from measured concentrations and 10-day averages of river discharge obtained from United States Geological Survey (USGS) and Tennessee Valley Authority (TVA) gaging stations. Input fluxes from tributaries and the upper Ohio River were compared with outputs near the confluence with the Mississippi to determine whether the Ohio River was a net source or sink for THMFP, chl *a*, and total organic carbon (TOC).

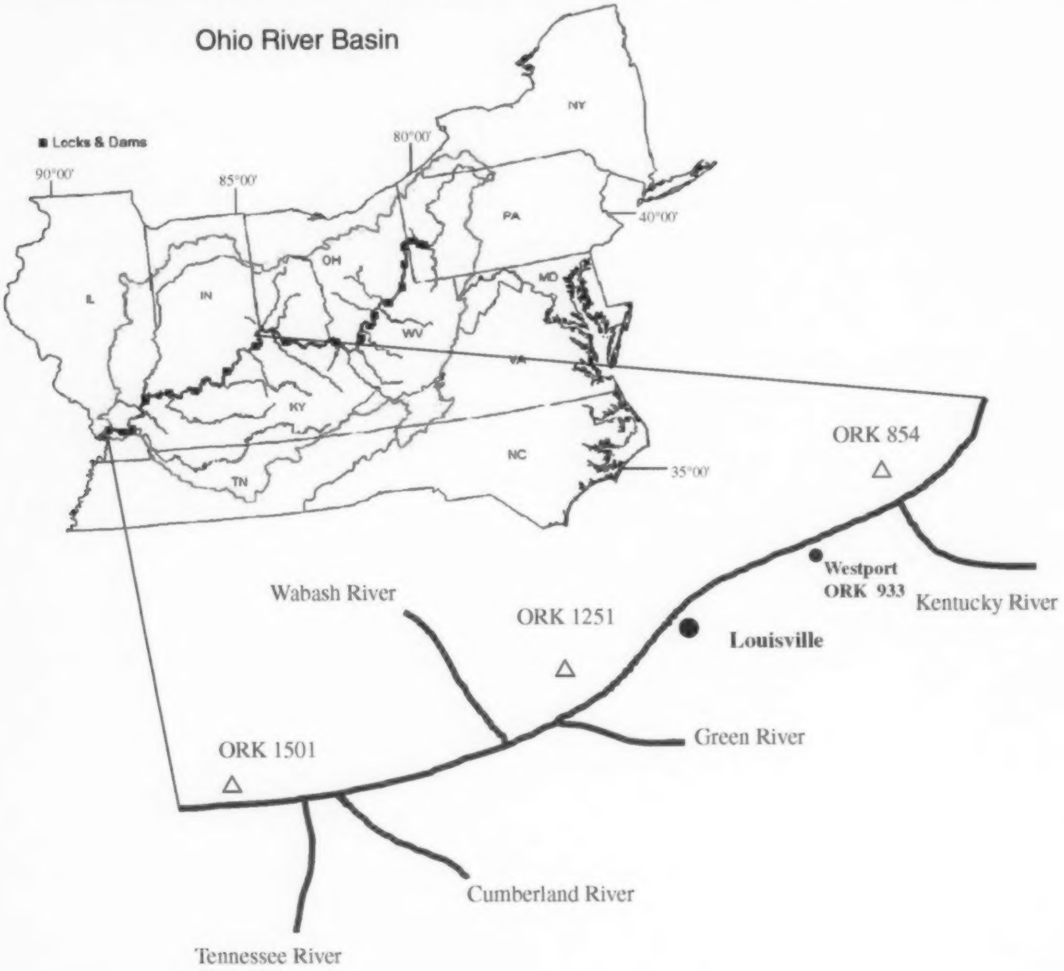
Experimental studies

Experiments designed to assess the effects of algal blooms on THMFP were conducted at the Ohio River Experimental Station located near Westport, Ky. (ORK 933). The facility consists of 24 large (2000-L) outdoor tanks that were filled with water pumped directly from the Ohio River. Three experiments were conducted in July, August, and September of 2000 to capture a range of conditions characteristic of the summer, base-flow period. Submersible pumps maintained particulate matter in suspension during the 6-day experiments. Polyvinyl chloride tank covers with varying aperture dimensions regulated light levels within the tanks, and thereby controlled algal bloom development. "Low" light tanks simulated the light environment occurring in deeper sections of the river (8- to 10-m average cross-sectional depth, chl *a* values $<5\ \mu g \cdot L^{-1}$) where net algal production is near zero; "high" light tanks simulated shallow reaches of the river (3- to 5-m depth, chl *a* values $10–15\ \mu g \cdot L^{-1}$) where higher algal growth rates result in greater bloom development (Sellers 2002). Three replicate high and low-light tanks were sampled on days 0, 3, and 6 during each experiment. In the September experiment, three additional tanks ("dark" treatment; zero aperture) were used to assess the effects of algal senescence on THMFP. Sample analyses for all treatments included determination of THMFP, chl *a*, POC, and DOC. Ancillary data (temperature, pH, turbidity, and dissolved oxygen) were collected to ensure that tank conditions mimicked those observed in the river.

Analytical procedures

An operationally defined procedure has been developed to quantify THMFP in samples incubated under standardized conditions (Method 5710B; APHA 1998). Both tank and river samples were collected in 1-L glass bottles and stored

Fig. 1. Locations of Ohio River sampling sites and five major tributaries, U.S.A. Inset map is modified from United States Army Corps of Engineers (available at http://f7784.lrh.usace.army.mil).



on ice in the dark for no more than 24 h. Samples were buffered to pH 7.0, chlorinated with an excess of free chlorine, and incubated at 25°C. Chlorine demand, dose, and free residual were determined according to Standard Methods (APHA 1998). After the 7-day reaction period, the samples were quenched with ascorbic acid. Concentrations of individual THMs (bromoform, bromodichloromethane, dibromochloromethane, and chloroform) were determined by the purge and trap gas chromatographic – mass spectrometric method (Method 6232C; APHA 1998). We report total THMFP as the sum of the four species.

Samples for chlorophyll analyses were collected on 0.5-µm filters, extracted in acetone (12 h), and analyzed by fluorometry (Turner Designs 10-AU, Sunnyvale, Calif.) with acid correction (Arar and Collins 1997). POC was collected on 0.5-µm filters, dried and analyzed using a Perkin-Elmer 2400 series II CHNS/O analyzer (Shelton, Conn.). Filtrate was analyzed for DOC on an automated total organic carbon analyzer (Shimadzu Model TOC-5050A, Kyoto, Japan) after sparging for 4 min to remove inorganic carbon. Temperature, pH, and dissolved oxygen were measured with a Hydrolab SOND IV (Austin, Tex.) and turbidity was measured with a Hach 2100P turbidimeter (Hach Co., Loveland, Colo.). Experimental data were analyzed by one-way analysis of variance (ANOVA) following tests for normality and homogeneity of variance using SYSTAT v. 7.0. Survey data were analyzed using univariate regressions to identify significant predictors of variation in THMFP across sites.

Results

Comparative studies

Drainage basin characteristics and C, N, and P concentrations for the Ohio River and its major tributaries are shown in Table 1. THMFP concentrations throughout the Ohio River basin ranged between 150 and 300 $\mu g \cdot L^{-1}$ (Fig. 2), with the exception of the Wabash River, which exhibited the highest

Table 1. Ohio River kilometre (ORK) denotes the location of mainstem sampling sites and for tributaries their confluence with the Ohio River.

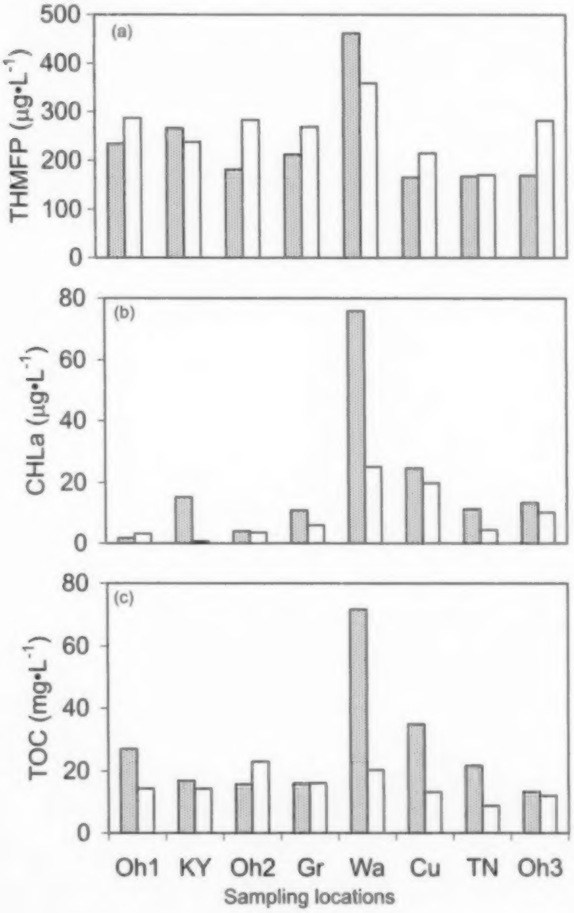
	ORK	Basin area (km^2)	Discharge (km^3·year^{-1})	Total N (µg·L^{-1})	Total P (µg·L^{-1})	Total OC (mg·L^{-1})
Ohio River (upper)	854	215 410	103.2[a]	1697	88	10.8
Kentucky River	879	18 052	7.6[a]	1030	89	9.1
Ohio River (middle)	1251	251 230	115.8[a]	398	90	11.4
Green River	1254	23 906	10.8[a]	1669	85	13.1
Wabash River	1356	85 729	25.0[a]	2805	219	24.6
Cumberland River	1472	46 413	33.2[a]	538	118	12.1
Tennessee River	1491	105 957	57.4[b]	521	73	8.7
Ohio River (lower)	1501	528 205	246.6[a]	1143	99	9.7

Note: Chemistry data are averages for August and September sampling dates in 1998, 1999, and 2000. OC, organic carbon.

[a] Data from Water Resources Data, Kentucky, Water Year 1997 (USGS KY-97-1) (http://ky.water.usgs.gov/).

[b] Data from Tennessee Valley Authority, Tennessee Water Management Office, Knoxville, Tenn.

Fig. 2. Concentrations of (*a*) trihalomethane formation potential (THMFP), (*b*) chlorophyll *a* (CHLa), and (*c*) total organic carbon (TOC) within the Ohio River and its major tributaries during August (shaded) and October (open) of 2000. Sampling sites were upper (Oh1), middle (Oh2), and lower Ohio (Oh3), Kentucky (KY), Green (Gr), Wabash (Wa), Cumberland (Cu), and Tennessee (TN) rivers.



THMFP concentrations during both the August (462 µg·L^{-1}) and the October surveys (359 µg·L^{-1}). The Wabash River also exhibited elevated chl *a* and TOC concentrations (Fig. 2). A univariate regression based on chl *a* accounted for 44% of the variation in THMFP, whereas a similar model using TOC as a predictor of THMFP accounted for 40% of variation. These models predicted comparable yields of THMFP per each unit increase in chl *a* (2.9 ± 0.9 µg·L^{-1}) or TOC (3.4 ± 1.1 µg·L^{-1}) over the range of chl *a* (1–25 µg·L^{-1}) and TOC (10–30 mg·L^{-1}) observed in our study. TOC was composed of nearly equal fractions of dissolved (45%) and particulate (55%) forms (average for all samples). Algal carbon (estimated as 30:1 C : chl *a*) was 4% of POC (range 1–7%), suggesting that riverine organic matter was predominantly of allochthonous origin.

Discharge from the Ohio River basin was similar during the two periods with, outputs averaging 3600 cm (August) and 3900 cm (October; Fig. 3). The relative contributions of various sub-basins differed between the two surveys. In August, the upper Ohio (39%) and Tennessee rivers (30%) dominated inputs with the Cumberland (15%), Wabash (10%), and Green–Kentucky rivers (5% combined), accounting for a smaller fraction of inflow. In October, the upper Ohio (36%) and Wabash (32%) rivers were the largest contributors, whereas the Tennessee (21%), Cumberland (10%), and Green–Kentucky rivers (1%) accounted for a smaller proportion of inputs. Material flux rates generally followed patterns in discharge although some differences were noted among sub-basins in their relative contributions of water and dissolved constituents (Fig. 3). In August, the Wabash River was a modest contributor of water (11% of inputs), but accounted for a disproportionately large fraction of THM precursors (22%), DOC (22%), and chl *a* (49%). A similar pattern was observed in October when the Wabash River accounted for 32% of water, 41% of THM precursors, 66% of chl *a*, and 45% of DOC inputs. In contrast, the upper Ohio River contributed similar proportions of water (39%), THMFP (40%), and DOC (35%) inputs, but accounted for only 4% of chl *a* inputs (values for August).

Total inflows to the study reach (from upper Ohio River and tributaries) were equivalent to 85 and 88% of the outflow volume during the August and October index periods, respectively. Observed declines in river stage (USGS data available at http://waterdata.usgs.gov/ky/nwis) during both

Fig. 3. Input and output fluxes of water, trihalomethane formation potential (THMFP), chlorophyll *a* (CHLa), dissolved organic carbon (DOC), and particulate organic carbon (POC) to the Ohio River during (*a*) August and (*b*) October of 2000. Input sources include the Tennessee (horizontal bars), Cumberland (dotted), Wabash (vertical bars), Kentucky + Green (cross-hatched), and Ohio (solid) rivers.

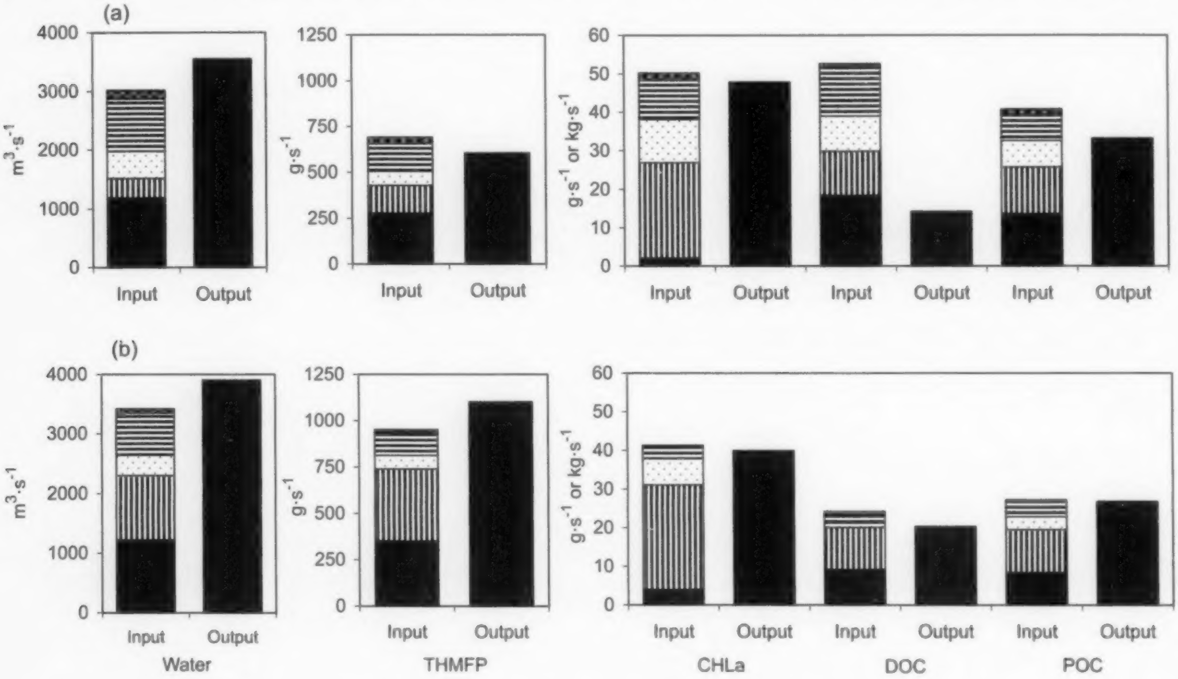


Table 2. Mean and range (in parentheses) of temperature, pH and dissolved oxygen (DO) in the Ohio River and experimental mesocosms during July, August, and September experiments.

Month	Location	Temperature (°C)	pH	DO (mg·L^{-1})
July	River	26.6 (25–27)	7.8 (7.6–8.0)	6.3 (6.2–6.4)
	High light	27.2 (26–29)	8.4 (8.2–8.9)	8.0 (7.9–8.0)
	Low light	27.1 (25–29)	8.3 (8.1–8.5)	7.3 (7.1–7.4)
August	River	27.1 (26–29)	7.8 (7.6–8.2)	6.1 (5.4–7.1)
	High light	26.5 (25–28)	8.5 (7.9–8.8)	7.5 (6.4–8.6)
	Low light	26.4 (25–28)	8.2 (7.9–8.4)	7.2 (6.4–8.0)
September	River	22.5 (20–25)	8.1 (7.8–8.5)	6.5 (5.7–7.3)
	High light	17.9 (15–25)	8.2 (8.2–8.4)	8.2 (7.2–9.2)
	Low light	17.7 (15–25)	8.3 (7.9–8.4)	8.1 (7.3–8.7)
	Dark	19.0 (15–25)	8.2 (8.1–8.2)	8.0 (7.1–8.6)

Note: Mesocosm data are shown for high-light, low-light, and dark (September only) treatments.

index periods suggest that surveys were conducted during a period of decreasing water storage. Therefore, we calculated retention estimates with a correction for the change in storage by assuming an outflow volume equivalent to the inflow volume. In August, input fluxes of THM precursors were 691 g·s^{-1}, while outputs (corrected for storage) were 514 g·s^{-1}. Retention estimates (as percentage of inputs) were 26% for THM precursors, 19% for chl *a*, 77% for DOC, and 31% for POC. In October, input fluxes of THMFP (951 g·s^{-1}) were comparable to output fluxes (964 g·s^{-1}), suggesting that the Ohio River was neither a net sink nor net source of THM precursors at this time. Retention estimates for chl *a* (15%), DOC (26%), and POC (14%) were also lower than those observed in August.

Experimental studies

Physical–chemical conditions in the mesocosms were comparable to concurrent river conditions (Table 2). Water temperature within the mesocosms was within 1°C of the river, except during the September experiment when cool nights resulted in more rapid heat loss from the tanks compared with the river. The pH of the mesocosms (8.2–8.4) was slightly higher than that of the river (7.8–8.1), particularly among tanks experiencing large algal blooms (high-

Fig. 4. Mean (± standard error) of chlorophyll concentrations on days 0, 3, and 6 in high-light (open) and low-light (shaded) tanks during (*a*) July, (*b*) August, and (*c*) September experiments. Asterisks denote significant differences between high- and low-light tanks. Concurrent river concentrations are shown for comparison.

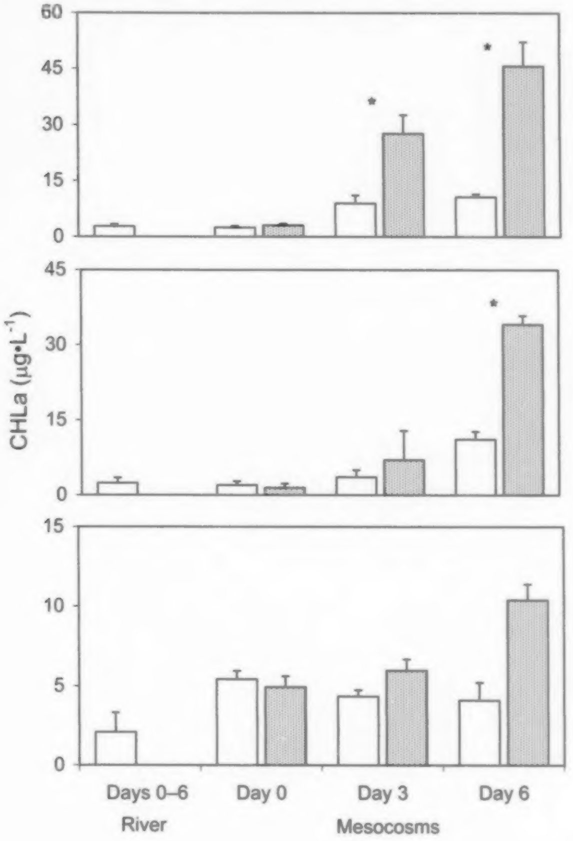
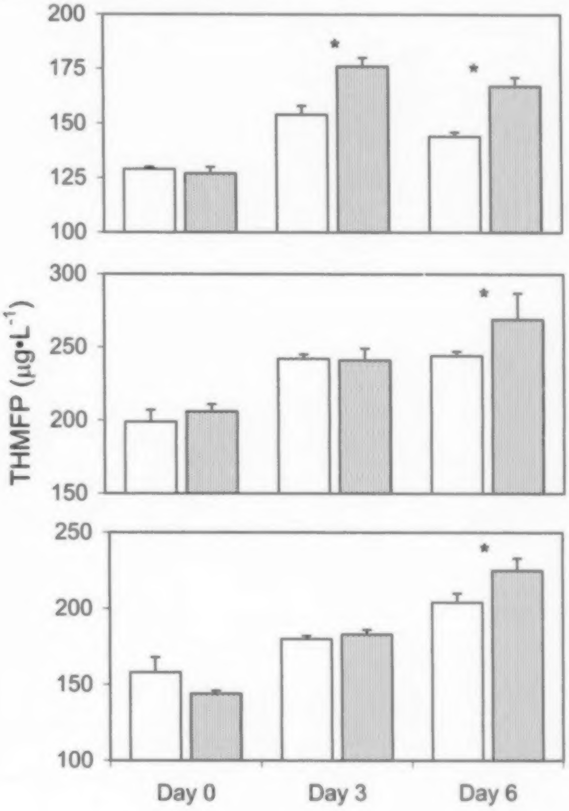


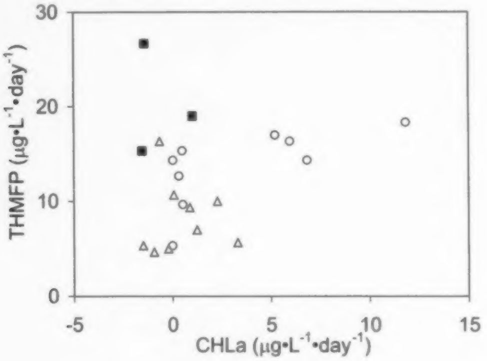
Fig. 5. Mean (± standard error) of trihalomethane formation potential (THMFP) on days 0, 3, and 6 in high-light (open) and low-light (shaded) tanks during (*a*) July, (*b*) August, and (*c*) September experiments. Asterisks denote significant differences between high- and low-light tanks.



light treatment; July and August experiments). Dissolved oxygen differences followed a similar trend with tank concentrations 1–1.5 mg·L^{-1} higher than the river and the largest differences occurring in high-light tanks. Variable shading produced the desired differences in algal bloom formation between high- and low-light tanks (Fig. 4). Differences in chlorophyll were apparent by day 3, and peak concentrations in high-light tanks (day 6) were three- to four-fold higher than those observed in low-light tanks. Low-light tanks exhibited modest gains in chlorophyll with peak concentrations (4–10 µg·L^{-1}) exceeding concurrent levels in the river (1–5 µg·L^{-1}). Although shading levels were held constant for all three experiments, differences in day length, cloud cover, and river turbidity resulted in variable light dosages and algal bloom formation among experiments. Highest light dosages and chlorophyll concentrations (ca. 45 µg·L^{-1}) occurred during the July experiment when water temperature was high (mean 27°C) and turbidity was low (mean 15 nephelometric turbidity units (NTU)). During the August experiment, water temperature was similar (mean 25°C), but turbidity was higher (mean 40 NTU), resulting in somewhat lower peak chlorophyll concentrations (30 mg·L^{-1}). Lowest chlorophyll concentrations (maximum 10 mg·L^{-1}) were observed during the September experiments when water temperature was lowest (mean 19°C). dark tanks (September experiment only) showed expected declines in algal abundance with chlorophyll decreasing from starting concentrations of 5 µg·L^{-1} to less than 1 µg·L^{-1} by day 6 (data not shown).

Initial (day-0) concentrations of THMFP among tanks ranged from 125 µg·L^{-1} (July) to 200 µg·L^{-1} (August) and were comparable to Ohio River concentrations during the August and October surveys (Figs. 2 and 5). Chloroform was always the dominant species, constituting >80% of the total THMFP. By comparing paired whole-water and filtered (0.5 µm) samples on three dates, we found that most of the THMFP (>91%) was associated with the dissolved fraction. THMFP concentrations increased in both high- and low-light tanks during all experiments. Larger increases were observed in high-light tanks (average 39% for three experiments) in comparison with low-light tanks (average 21%). By day 6, differences were statistically significant (p < 0.05) in all

Fig. 6. Rates of change in chlorophyll and trihalomethane formation potential (THMFP) between days 0 and 6 among high-light (○), low-light (△), and dark (■) tanks. Symbols denote individual tanks ($N = 3$) from July, August, and September experiments (dark tanks September only).



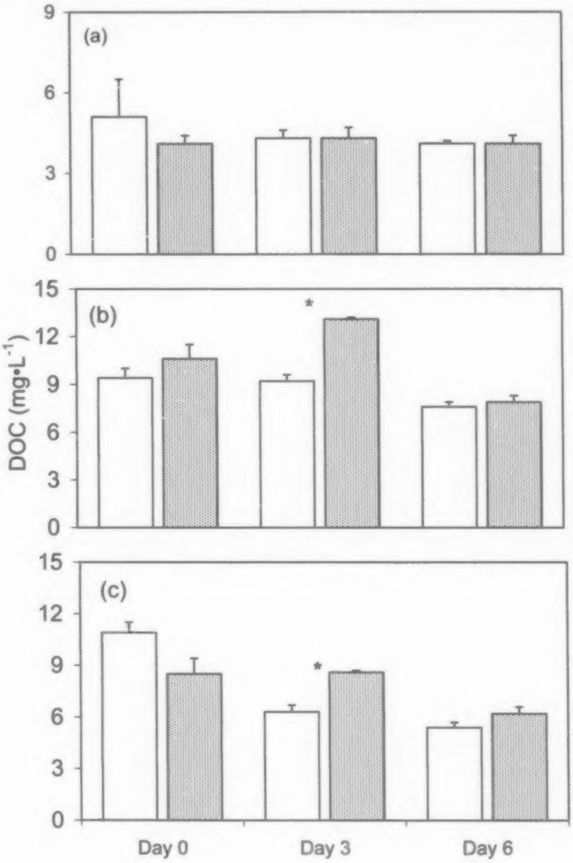
three experiments with high-light tanks exceeding low-light tanks by 20–25 µg THMFP·L^{-1}. Within each experiment, increases in THMFP generally followed increases in chl *a*, but comparisons of data from the three experiments did not reveal a consistent, linear relationship between chl *a* production and generation of THM precursors (Fig. 6). High-light tanks exhibiting large algal blooms (July and August experiments; chl $a > 5$ µg·L^{-1}·day^{-1}) also exhibited large increases in THMFP (14–18 µg·L^{-1}·day^{-1}). Among high- and low-light tanks exhibiting smaller blooms, changes in THMFP were variable (5–15 µg·L^{-1}·day^{-1}) but generally lower (mean 9 µg·L^{-1}·day^{-1}). The largest increases in THMFP (mean ± standard error 47 ± 18 µg·L^{-1}·day^{-1}) were observed among the three dark tanks (September experiment) that exhibited low or negative rates of change in chl *a*.

Variable tank shading and algal bloom development did not result in consistent differences in tank DOC concentrations (Fig. 7). Final (day-6) concentrations were not significantly different between high- and low-light tanks in any of the three experiments.

Transient effects were noted on day 3 of the August and September experiments when DOC concentrations differed by 3 mg·L^{-1} between high- and low-light tanks. DOC concentrations were low (ca. 5 mg·L^{-1}) during the July experiment and higher (ca. 10 mg·L^{-1}) during the August and September experiments. DOC concentrations generally declined during these two experiments from starting concentrations near 10 to 5–8 mg·L^{-1}. Rates of change in DOC among individual tanks were not correlated with changes in THMFP or chlorophyll.

Previous studies have calculated THM yields as a function of DOC or TOC (Graham et al. 1998; Stepczuk et al. 1998*a*; Galapate et al. 1999). Since the majority of our THMFP originated in the dissolved fraction, we derived THM yields based on DOC (µg THM·mg^{-1} DOC). In our river surveys, THM yields ranged from 8 to 65 µg THM·mg^{-1} DOC across all sites. On average, yields were higher among October samples compared to August (40 vs. 25 µg THM·mg^{-1} DOC, respectively; paired *t* test, $p = 0.06$). THM yields among mesocosms ranged from 10 to 54 µg THM·mg^{-1} DOC (mean 34 µg THM·mg^{-1} DOC) and were similar during July, August, and September experiments. There was no significant difference in THM yield between high- and low-light treatments (July and August experiments; paired *t* tests, $p > 0.44$) or among the low-light, high-light, and dark treatments (September experiment; ANOVA $p = 0.48$). THM yields increased from day 0 to day 6 because THMFP increased while DOC concentrations remained stable.

Fig. 7. Mean (± standard error) of dissolved organic carbon concentrations on days 0, 3, and 6 in high-light (unshaded) and low-light (shaded) tanks during July, August, and September experiments. Asterisks denote significant differences between high- and low-light tanks.



Discussion

Our survey revealed a two-fold range of variation in THMFP among large rivers composing the Ohio River basin. THM concentrations were comparable to those reported for other rivers and reservoirs (Veenstra and Schnoor 1980; Graham et al. 1998; Stepczuk et al. 1998*a*). Most of the THMFP (>91%) was associated with the dissolved fraction and chloroform was always the dominant species, as has been reported in prior studies (Graham et al. 1998; Stepczuk et al. 1998*a*, 1998*b*). Previous studies have also assessed THMFP yield from organic carbon by comparing ratios of THMFP/DOC (Galapate et al. 1999; Stepczuk et al. 1998*a*), THMFP/TOC (Graham et al. 1998), or comparing chloroform produced by algal cultures normalized to moles of available halides and organic carbon (Watcher and Andelman 1984). Unfortunately, the latter three studies all used shorter incubation periods (24 h) than is currently recommended (APHA 1998). It would be inappropriate to compare yields among these experiments, since it is well established that THMFP is affected by contact time with chlorine, as well as other factors. Stepczuk et al. (1998*a*) used 7-day incubations (as in our study) and reported an average yield of 119 μg THMFP·mg^{-1} DOC. Our yields of THMFP (<50 μg THM·mg^{-1} DOC) were 50% lower and may reflect watershed-level differences in inputs of allochthonous (terrestrially derived) precursors or internal rates of precursor formation. Previous studies have also reported that THM yields from DOC can vary tremendously, particularly among lotic systems (Veenstra and Schnoor 1980).

Our field and experimental data were generally supportive of the hypothesis that algal production enhances THM formation. The highest THMFP concentrations were found in the Wabash River that also exhibited the highest chlorophyll concentrations. In manipulative experiments, tanks exhibiting large algal blooms showed large increases in THMFP. Yields of THMFP within mesocosms (2.4 ± 0.4 μg THMFP per microgram chl *a*) were comparable to those obtained from survey data (2.9 ± 0.9 microgram THMFP per microgram chl *a*). To our knowledge, this is the first study to establish a correlative linkage between THM formation and algal abundance in both experimental and environmental settings. A positive correlation between chl *a* and THMs has also been reported for reservoirs in England (Graham et al. 1998). In contrast, Stepczuk et al. (1998*a*) found no relationship between THMFP and chlorophyll concentrations in the West Branch of the Delaware River. Variation in the strength of the chl *a* – THM relationship may be attributed in part to differences in the importance of autochthonous production among the systems investigated. Riverine carbon sources are dominated by allochthonous inputs of detrital material, but our survey data suggest that conditions that favor algal bloom development are also associated with high THMFP (as in the Wabash River). Chlorophyll concentrations within the Ohio River and its tributaries were within the range reported for large rivers (Admiraal et al. 1990; Basu and Pick 1995; Spaink et al. 1998), suggesting that autochthonous sources of THM precursors may be important in other rivers. Furthermore, our experimental data show that even modest increases in chl *a* (5–25 μg·L^{-1}) result in 40–50% increases in THMFP. It is interesting to note that although concentrations of chlorophyll and THM precursors were correlated in our survey data set, their flux rates were not. In October, input and output fluxes of THM precursors were higher than those observed in August, despite generally lower chlorophyll fluxes. This pattern arises from seasonal changes in the relative importance of inflows among the major tributaries of the Ohio River. Greater THMFP inputs during October were largely due to elevated fluxes from the Wabash River, while reductions in chl *a* inputs were due to reduced inflows from the Tennessee and Cumberland rivers. These data suggest that the relative importance of autochthonous and allochthonous sources of THM precursors varies among the tributaries and may account for a substantive fraction of the unexplained variation in our chl *a* – THMFP model. Although our limited temporal resolution precludes an adequate assessment of inter-basin differences, these findings suggest that seasonal changes in hydrologic routing within the Ohio River basin may have an appreciable influence on downstream THMFP.

Our experimental data demonstrate a positive association between algal growth and THMFP, but comparisons across experiments show that the generation of THM precursors did not follow a linear relationship with chl *a* production. These findings suggest that multiple mechanisms may act to influence THMFP in our mesocosms including algal growth and senescence as well as photolytic effects on ambient DOC pools. Graham et al. (1998) reported that THM yields from both actively growing and senescent algal cultures were greater than those of cultures in the stationary phase. Others have suggested that ECPs released during algal growth may yield greater quantities of THM precursors than the subsequent decomposition of algal cells (Watcher and Andelman 1984). We derived inferred values of ECP production to assess their potential importance during our mesocosm studies. Chlorophyll concentrations at the start and end of each experiment were converted to C units (based on 30:1 C : chl *a*) and used to estimate net production of algal C during the 6-day experiment. Release of ECPs was estimated as 13% of algal production (Baines and Pace 1991). Among high-light tanks during the July and August experiments, the amount of C released as ECP (127 and 167 μg C·L^{-1}, respectively) exceeded by more than 20-fold the amount of C required to produce the observed increase in THM as chloroform, which was the dominant species of THM in our study rivers (>84%). Since C composes only 10% of the mass of THMs (when chloroform predominates), the formation of THMs represented a very small C demand (4 μgC·L^{-1} and 6 μgC·L^{-1}, respectively) relative to ECP production. Among tanks exhibiting lower rates of algal growth, THM formation corresponded to 5–38% of C released as ECPs. C demand for THM formation exceeded ECP release only among low-light tanks during the September experiment (when net algal production was lowest). These analyses suggest that extracellular release of photosynthate was sufficient to account for the observed changes in THMFP, though current methodology does not enable us to partition ECPs vs. diagenetic sources of THM precursors.

Large increases in THMFP occurred in our dark tanks (September experiment only) suggesting that algal senescence and subsequent release of DOC is a source of THM precursors in low-light environments. This process may be particularly important in large rivers, unstratified reservoirs, and the hypolimnia of lakes and reservoirs where unfavorable light conditions result in decompositional environments. We estimated potential C fluxes arising from algal senescence based on measured declines in chl *a* (30:1 C : chl *a*) and found that these were seven-fold higher than the C required to account for the observed increases in THM. Much of this algal-derived C is labile and therefore is likely to be respired. With correction for respiration losses (assuming 15% bacterial growth efficiency), C release from algal biomass still exceeded C demand for THM formation by two-fold. These analyses underscore the difficulty of tracking sources of THM precursors since they represent a small proportion of potential fluxes associated with algal growth and senescence and an even smaller fraction (<0.1%) of the background pool of DOC.

For tanks exhibiting modest changes in chlorophyll ($<5\ \mu g \cdot L^{-1} \cdot day^{-1}$), increases in THMFP were generally higher among high-light tanks than low-light tanks. Therefore, we cannot exclude the possibility that solar radiation has direct effects on THMFP. Solar radiation in the ultraviolet (UV) range has been shown to cause photolysis of certain types of DOC (Moran and Zepp 1997; Opsahl and Benner 1998) that could result in the production of THM precursors. Li et al. (1996) found that TOC decreased and the yield of chloroform and total THMs from a humic acid sample increased after irradiation at a wavelength of 253.7 nm. Light attenuation in the Ohio River and its tributaries is regulated by suspended particulate materials and varies seasonally with discharge. Penetration of UV light into the water column is limited compared with other systems like low DOC lakes (Williamson et al. 1999; Bukaveckas and Robbins-Forbes 2000). However, during the period of summer base flow (typically July–October), turbidity is at its lowest point of the year (<7 NTU) and conditions may be favorable for photolysis, particularly in shallower reaches. Further study is needed to resolve the possible role of UV effects on the production of THM precursors.

Increases in THMFP among high- and low-light and dark tanks suggest that algal production, algal senescence, and photolysis may all play a role in the internal generation of THM precursors in large rivers. These processes were of sufficient magnitude to increase THMFP by as much as 50% over 3–6 days within our experimental mesocosms. Their importance in situ will depend on rates of biotic and photolytic activity as well as water residence time in the rivers, which varies (e.g., from <1 to >20 days for the McAlpine Pool) depending on discharge. The net effect of riverine processes on precursor production and loss can be inferred from comparisons of inputs and outputs. In August, tributary and upstream inputs of THMFP as well as chl *a*, DOC, and POC exceeded outputs from the Ohio River. Differences between input and output fluxes were larger (e.g., 26% for THMFP) than the error typically ascribed to measurements of discharge and solute concentration (10–15%). Our estimation of retention was based on the assumption that the observed imbalances in the water budget (<20%) were attributable to decreases in channel storage coinciding with falling river stage. We cannot discount the possibility that underestimation of total inputs due to small tributaries and other ungauged sources may contribute to this imbalance. We estimated additional inputs from these sources assuming an inflow volume equivalent to that required for balancing the water budget and concentrations derived from volume-weighted averages for the measured inflows. Retention derived by this method yielded a similar estimate (e.g., 27% for THMFP) to that calculated based on a correction for changing storage. We conclude that at this time, the river was acting as a net sink for THM precursors, dissolved organic matter, and algal C. These patterns are indicative of a predominance of bacterial respiration over photosynthesis that is generally characteristic of river and reservoir environments (Benner et al. 1995; Howarth et al. 1996; Bukaveckas et al. 2002). By October, water temperatures were lower and the associated decline in algal and bacterial activity may account for the river being in equilibrium with respect to inputs and outputs of THMFP, chl *a*, and DOC. The Wabash River accounted for a large fraction of THMFP and chl *a* inputs during October, and since its confluence with the Ohio River is near the downstream end of our study reach, short residence time may have also contributed to low retention. Overall, our findings suggest riverine processes may attenuate THMFP and thereby benefit water quality downstream, but that these effects are seasonally variable. More detailed studies are needed to quantify the importance of these processes on an annualized basis, since our data are based on low flow conditions.

Our results indicate that water utilities may face considerable challenges as they prepare strategies to meet the new THM guidelines from the USEPA. First, it is clear that there are watershed-level differences in THMFP among source waters within the Ohio River basin that may make it difficult for utilities in the region to adopt a uniform strategy for reducing THMs. For example, water companies whose intakes draw from the Wabash River face greater challenges in meeting THM guidelines than plants located along the Kentucky or Green rivers. Similarly, the instream processes that make the Ohio River a sink for THMFP could benefit utilities located downstream, while those further upstream may have to be more aggressive in their approach to the management of THM. Secondly, the importance of photosynthetically derived THM precursors highlights the need for continued efforts to mitigate algal blooms in water bodies receiving anthropogenic nutrient inputs. Traditional approaches have relied on in-plant solutions such as removal of algae through coagulation and flocculation, but these may not reduce concentrations of algal ECPs (El-Dib and Ali 1994; Graham et al. 1998). Prechlorination of intake waters may increase levels of THM precursors as the contents of the lysed algal cells become available to form disinfection by-products. As regulations for THM levels in finished water become more stringent, such narrowly focused solutions may no longer be economically attractive and greater attention needs to be directed to the ecological processes in rivers and other source waters that determine THMFP. Improved understanding of both watershed-level and insteam processes that influence THMFP would help utilities to develop more innovative and cost-effective ways of meeting their obligation to regulatory agencies and customers.

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Reference priors for Bayesian fisheries models

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Abstract: Bayesian models require the specification of prior distributions for all unknown parameters, and this formal utilization of prior knowledge (if any) can be used to great advantage in some fisheries. However, regardless of whether prior knowledge about model parameters is available, specification of prior distributions is seldom unequivocal. This work addresses the problem of specifying default priors for several common fisheries models. To maintain consistency of terminology with the statistical literature, such priors are herein called reference priors to recognize that they can be interpreted as providing a sensible reference point against which the implications of alternative priors can be compared. Here, the Jeffreys' prior is demonstrated for the Ricker and Beverton–Holt stock–recruit curves, von Bertalanffy growth curve, Schaefer surplus production model, and sequential population analysis. The Jeffreys' priors for relevant derived parameters are demonstrated, including the steepness parameter of the Beverton–Holt stock–recruit curve. The sequential population analysis example is used to show that the Jeffreys' prior should not be automatically accepted as a reference prior in all models—this needs to be decided on a case-by-case basis.

Résumé : Les modèles bayésiens exigent que l'on spécifie des distributions a priori pour tous les paramètres inconnus et cette utilisation formelle de données a priori (lorsqu'elles existent) est d'une grande utilité dans l'étude de certaines pêches commerciales. Cependant, qu'il y ait ou non des informations a priori sur les paramètres du modèle, l'attribution des distributions a priori est rarement sans équivoque. On trouvera ici un examen du problème d'assigner des distributions a priori dans plusieurs modèles courants utilisés en halieutique. Pour maintenir une uniformité de terminologie avec la littérature statistique, ces distributions à priori sont appelées distributions a priori de référence pour indiquer qu'elles peuvent procurer un point de référence crédible auquel les caractéristiques d'autres distributions a priori de rechange peuvent être comparées. La distribution a priori de Jeffreys est déterminée pour les courbes de stock–recrutement de Ricker et de Beverton–Holt, la courbe de croissance de von Bertalanffy, le modèle de production excédentaire de Schaefer et l'analyse séquentielle de population. Les distributions a priori des paramètres dérivés pertinents sont aussi explicitées, y compris le paramètre d'inclinaison de la courbe stock–recrutement de Beverton–Holt. L'exemple de l'analyse séquentielle de population sert à démontrer que la distribution de Jeffreys ne doit pas être retenue automatiquement comme référence pour tous les modèles—c'est un choix à faire cas par cas.

[Traduit par la Rédaction]

Introduction

In the context of fisheries science, Bayesian modeling has been promoted as a methodology for realistically capturing the uncertainties and resulting risks inherent to a fishery (Punt and Hilborn 1997). Fundamental to this is the need to specify a prior distribution to represent the information (if any) that exists about unknown parameters prior to collection of the data, and the current popularity of Bayesian models in fisheries owes much to the recognition by fisheries scientists that substantial prior information often does exist. For example, Hilborn and Liermann (1998) show how to use meta-analysis to use existing information from studies on related stocks.

In the absence of prior knowledge it has been common practice to seek an appropriate "noninformative" prior distribution. It is well known that flat priors are not noninformative (in general) and considerable effort has been devoted to obtaining general purpose methods for constructing priors that express the state of ignorance. This pursuit has resulted in intense controversy because different axiomatic assumptions can result in different constructions for "noninformative" priors (Kass and Wasserman 1996).

The existence of prior information does not eliminate the potential controversy associated with specification of a prior distribution. There are likely to be contestable assumptions made concerning the inclusion and exclusion of existing information and in its transformation from prior information into a statistical prior distribution. Moreover, when studies on related stocks are available then care must be taken to apply meta-analysis only to those parameters satisfying the underlying assumptions of this approach. Meta-analysis typically requires the parameters to be exchangeable, which in this context requires that they can be reasonably modeled as having a common distribution over all stocks considered. This may be appropriate for parameters such as catchability, natural mortality, and recruitment depensation (Hilborn and Liermann 1998), but would not be appropriate for stock-specific parameters like virgin biomass. Furthermore, the meta-analysis may itself depend on some assumed underlying hierarchical model, the specification of which could be contestable.

A recent viewpoint being adopted by an increasing number of Bayesians is to establish default or reference prior(s)

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for any given model. These priors are not presented as formal expressions of ignorance, but rather, as default priors that are sensible to use when prior information is not being used (Kass and Wasserman 1996). In this sense they provide a point of reference against which the consequences of using alternative priors can be compared. Herein, the construction of a catalog of potential reference priors for fisheries models is initiated.

The search for reference priors is, however, far from clear cut. Kass and Wasserman's (1996) review article gives details on more than a dozen formal methods for deriving priors, none of which are guaranteed to produce good reference priors. Of these many different methods, they do give special emphasis to the Jeffreys' method and show that several of the other methods produce reference priors similar to Jeffreys'. Jeffreys' method is by far the most widely used approach for obtaining reference priors and has previously been used in Bayesian fisheries modeling (e.g., Hoenig et al. 1994).

The dominance of Jeffreys' method for elicitation of potential default priors is due to its property of parameterization invariance. That inference should not depend on the particular, possibly arbitrary, choice of model parameterization is generally considered the most fundamental requirement of a reference prior. Alternatives to the Jeffreys' method include approaches based on the concepts of group invariance and missing information (Kass and Wasserman 1996). The group invariance method seeks a prior that preserves the statistical model under group transformations of the data, and the missing information method seeks a prior that maximizes the expected information in the data (as measured by the Kullback–Leibler distance between prior and posterior). Both of these alternatives require sophisticated mathematical tools (e.g., group and measure theory, limits, topology) and for many models it can require great mathematical literacy to simply comprehend the resulting prior. It is interesting to note that the Jeffreys' prior arises as a special case of these two alternatives (Kass and Wasserman 1996). In particular, under the group invariance method the Jeffreys' prior corresponds to the left Haar measure prior.

Here, the Jeffreys' method is employed throughout. Jeffreys' method is introduced in the next section, and in the following section is applied to the Ricker and Beverton–Holt stock–recruit curves, the von Bertalanffy growth curve, and the Schaefer surplus production model, as well as to sequential population analysis. The Discussion notes that the Jeffreys' priors obtained for the first four models appear to be reasonable for use as reference priors, but not so for the sequential population model.

Jeffreys' method

Parameterization invariance

The particular form of parameterization used in a model can be somewhat arbitrary, or may be driven by numerical considerations (e.g., Ratkowsky 1986). For example, from Hilborn and Walters (1992) and Quinn and Deriso (1999), the Beverton–Holt stock–recruit curve appears in the following four forms:

$$R = \frac{aS}{b+S} = \frac{a^* S}{1+\frac{a^*}{b^*}S} = \frac{\alpha S}{1+\beta S} = \frac{S}{\alpha^* + \beta^* S}$$

where R denotes recruitment and S denotes spawner abundance or biomass. The primary motivation for Jeffreys' method is the desire that inference should not depend on the particular parameterization of the model.

More generally, if a model is expressed in terms of parameters θ, then it can also be expressed in terms of parameters $\zeta = g(\theta)$, where g is any invertible transformation. If a formal method for constructing a prior distribution is to be employed then there are two different, but equally valid, ways that it can be implemented to obtain the prior on ζ: (*i*) Use the model expressed in terms of θ to obtain the prior for θ, and then apply the transformation of random variables formula (Freund 1992)

$$(1) \qquad \pi(\zeta) = \pi(\theta(\zeta))\left|\det\left(\frac{\partial\theta}{\partial\zeta}\right)\right|$$

where $\frac{\partial\theta}{\partial\zeta}$ is the Jacobian matrix with i, jth element $\frac{\partial\theta_i}{\partial\zeta_j}$. (*ii*) Express the model in terms of ζ and apply the method for constructing the prior (for ζ) to this model. Jeffreys' method is termed parameterization invariant because these two approaches result in the same prior for ζ

Jeffreys' prior

Jeffreys (1961) used measure-theoretic concepts to motivate his choice of a parameterization invariant prior. Jeffreys' prior is obtained as the square root of the determinant of the information matrix obtained from the likelihood function for the data. That is, denoting the parameters by $\theta = (\theta_1, \theta_2, \ldots, \theta_p)$ and the log-likelihood by $l(\theta)$, $I(\theta)$ is the $p \times p$ information matrix with i, jth element

$$I_{ij}(\theta) = E\left[\frac{\partial l(\theta)}{\partial\theta_i}\frac{\partial l(\theta)}{\partial\theta_j}\right]$$

and Jeffreys' prior is given by

$$(2) \qquad \pi(\theta) \propto \det(I(\theta))^{1/2}$$

A priori independence

Jeffreys (1961) noted that eq. 2 should not be applied verbatim in all situations, but rather, could be used to help establish conventions for representation of prior ignorance. One convention that has been justified on several grounds (Jeffreys 1961; Box and Tiao 1973; Kass and Wasserman 1996) is that variance (or standard deviation) parameters be excluded from the parameter vector θ and that eq. 2 be applied separately to θ and the variance parameters. This convention is often justified on the grounds that variance parameters can be presumed a priori independent from other parameters under the state of prior ignorance. Sun and Berger (1998) provide a formalization of this notion.

Fisheries models

Jeffreys' prior depends on the likelihood function for the parameters, and so will depend on the assumed form of the error terms. For example, the Jeffreys' prior on the asymptotic length parameter of a von Bertalanffy growth curve will depend on whether the length data are assumed to be normally or lognormally distributed. This is justifiable if one considers that the specification of the error term implicitly provides information about the parameters. A von Bertalanffy growth model that puts normal error on the observed lengths is theoretically permitting them to be negative, and so it is not unreasonable that a "noninformative" prior would put positive prior probability on asymptotic length being negative. This would not occur with lognormal errors.

In the five models considered below, the data are necessarily nonnegative and lognormal error has been assumed throughout. Consequently, separate application of eq. 2 to the standard error, σ, of the logged data gives the familiar reference prior (e.g., Box and Tiao 1973)

$$(3) \qquad \pi(\sigma) = 1/\sigma$$

This is equivalent to log(σ) having a flat prior (from eq. 1).

Here, the "standard" forms of the models have been used. For example, in the stock–recruit models there are the considerations of autocorrelated recruitments and measurement error in estimation of spawners (Ludwig and Walters 1981). These considerations are not covered here and the number of spawners is treated as a known explanatory variable. In the Schaefer surplus production model, there is the consideration of process error (Meyer and Millar 1999; Millar and Meyer 2000); however, here the more traditional observation error only model (Polacheck et al. 1993) is used.

The model descriptions and resulting Jeffreys' priors are given below for the five fisheries models. The parameterization invariance of Jeffreys' method ensures that the results do not depend on the particular model parameterizations used below. The derivations of the Jeffreys' priors are found in Appendix A.

Ricker stock–recruit curve (*a*,*b*)

Let S_t be the spawners at time t and let R_t be the measured number of recruits resulting from spawning at time t. The Ricker stock–recruit model with multiplicative lognormal errors is

$$(4) \qquad R_t = aS_t e^{-bS_t} e^{\nu_t}, \quad a > 0,\ b \ge 0$$

where ν_t are independent and identically distributed $N(0, \sigma^2)$ random errors.

The Jeffreys' prior for the above model is

$$\pi(a,b) \propto \frac{1}{a}, \quad a > 0,\ b \ge 0$$

That is, the Jeffreys' prior for a is uniform on the log scale and for b is uniform on $[0, \infty)$.

Beverton–Holt stock–recruit curve (a,*b*)

Using the same notation as in the previous model, the Beverton–Holt stock–recruit model with multiplicative lognormal errors is

$$(5) \qquad R_t = \frac{aS_t}{1 + bS_t} e^{\nu_t}, \quad a > 0, b \ge 0$$

The Jeffreys' prior is

$$\pi(a, b) \propto \frac{\mathrm{sd}(y(b))}{a}, \quad a > 0, b \ge 0$$

where $y_t(b) = S_t/(1 + bS_t)$ and sd($y(b)$) is the standard deviation of $y_t(b)$, $t = 1,\ldots,n$. That is,

$$\mathrm{sd}(y(b)) = \left(\frac{\sum_t (y_t(b) - \bar{y}(b))^2}{n - 1} \right)^{1/2}$$

Remarks

The Jeffreys' prior for b

$$(6) \qquad \pi(b) \propto \mathrm{sd}(y(b))$$

is proper (Appendix A). That is, with the appropriate normalizing constant it integrates to unity and corresponds to a proper density function.

For the purposes of meta-analysis of stock–recruit relationships, the Beverton–Holt stock–recruit model must be reparameterized in a form which permits a parameter that can reasonably be considered exchangeable over related stocks. Some authors (e.g., Hilborn and Liermann 1998) recommend expressing the Beverton–Holt curve in terms of a (in eq. 5) and a "steepness" parameter, h_ρ. Specifically, if S_0 is virgin number of spawners, then for ρ between zero and unity,

$$(7) \qquad h_\rho = \frac{R(\rho S_0)}{R(S_0)}$$

where $R(S) = aS/(1 + bS)$. Note that, in the absence of knowledge about spawners-per-recruit, S_0 can be prespecified independently of a and b. It is common practice to take $\rho = 0.2$, which gives $h_{0.2}$ the interpretation of being the ratio of recruitment at 20% of virgin numbers to recruitment in the virgin state.

It is necessarily the case that $\rho \le h_\rho \le 1$. If h_ρ is close to ρ, then recruitment decreases in a near-linear relationship with spawners (as spawners are reduced in number from S_0 to ρS_0), and if h_ρ is close to unity, then recruitment is little reduced by the reduction in spawners.

The Jeffreys' prior for steepness is obtained from applying the change of variables technique to $\pi(b)$. This gives (Appendix A)

$$\begin{aligned} \pi(h_\rho) &= \frac{1-\rho}{\rho S_0(1 - h_\rho)^2}\pi(b) \\ &= \frac{1-\rho}{\rho S_0(1 - h_\rho)^2}\mathrm{sd}(y(b(h_\rho))), \quad \rho \le h_\rho < 1 \end{aligned}$$

where

$$b(h_\rho) = \frac{h_\rho - \rho}{\rho S_0(1 - h_\rho)}$$

Example

Equation 6 was applied to the Alaska pink salmon escapement (*Oncorhynchus gorbuscha*, spawners) values (Fig. 1) from Quinn and Deriso (1999). The Jeffreys' prior for b (Fig. 2) has a maximum of sd(S) = 1.436 at $b = 0$ (Appendix A).

The Jeffreys' prior for the steepness parameter was calculated for four different specified values of the virgin number of spawners, 5, 10, 20, and 50 million fish, respectively (Fig. 3). It puts increasingly high prior density on high values of steepness (corresponding to a lesser decrease in recruitment due to a reduction in spawners) as S_0 increases. The direction of this relationship can be explained by noting that the virgin number of spawners, S_0, is irrelevant to the fitting of Beverton–Holt stock–recruit curves. It is merely a user-defined constant that is required to express h_ρ as a function of parameter b (eq. 15, Appendix A), and eq. 15 is an increasing function with respect to increasing S_0.

von Bertalanffy growth curve (L_∞, k, t_0)

Let a_i denote the age of fish i and let L_{a_i} denote its observed length. The von Bertalanffy growth model with multiplicative lognormal errors is

$$(8)\qquad L_{a_i} = L_\infty(1 - e^{-k(a_i - t_0)})e^{v_i}, \qquad L_\infty > 0, k > 0, t_0 < \min(a)$$

where L_∞ is asymptotic length, k is the growth rate, t_0 is the time at which expected length is zero, and v_i are independent and identically distributed $N(0, \sigma^2)$ random errors.

The Jeffreys' prior $\pi(L_\infty, k, t_0)$ cannot be written in a compact form because of the summations in the terms of the information matrix (Appendix A). However, it can be written in the form

$$(9)\qquad \pi(L_\infty, k, t_0) \propto \frac{1}{L_\infty}\pi(k, t_0), \qquad L_\infty > 0, k > 0, t_0 < \min(a)$$

That is, the Jeffreys' prior for L_∞ is uniform on the log scale and is independent of the joint Jeffreys' prior for k and t_0.

Example

The Jeffreys' prior density $\pi(k, t_0)$ from eq. 9 was calculated for the rougheye rockfish (*Sebastes aleutianus) age values from Quinn and Deriso (1999). It increases as k* decreases or t_0 increases (Fig. 4).

Quinn and Deriso (1999) assumed additive normal errors in their fit of the von Bertalanffy growth curve to these data. By analogous calculations to those shown in Appendix A, this model also results in prior independence of L_∞ and (k, t_0), but with $\pi(L_\infty) \propto L^2_\infty$. Also, $\pi(k, t_0)$ increases with increasing t_0, but is unimodal in k (Fig. 4).

Schaefer surplus production model (Q, K, r)

Hoenig et al. (1994) used the equilibrium Schaefer surplus production model and calculated the Jeffreys' prior for the parameter corresponding to optimal effort. Hilborn and Walters (1992) and Polacheck et al. (1993) demonstrate serious inadequacies with the equilibrium model and recommend use of the observation error version of the dynamic surplus production model. This model is presented below.

Letting B_y denote the biomass at the start of year y, and C_y denote the catch in year y, the dynamic Schaefer surplus pro-

Fig. 1. Alaska pink salmon (*Oncorhynchus gorbuscha*) escapement (spawners) and recruitment data from p. 105 of Quinn and Deriso (1999).

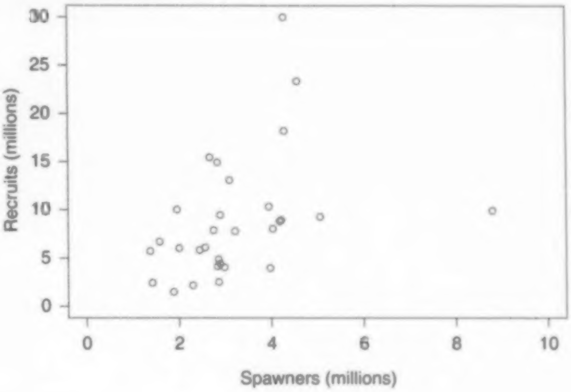


Fig. 2. Reference prior for parameter b of the Beverton–Holt stock–recruit model, calculated for the spawner data of Fig. 1.

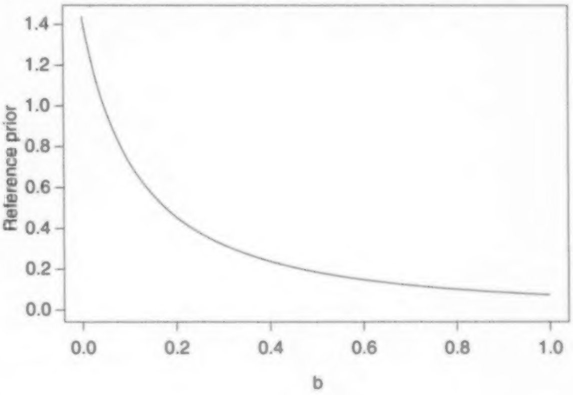


Fig. 3. Reference priors for the steepness parameter of the Beverton–Holt stock–recruit model, calculated for the spawner data of Fig. 1 for various S_0 values. S_0 = 5 (broken line), S_0 = 10 (solid line), S_0 = 20 (dotted line), S_0 = 50 (dotted broken line).

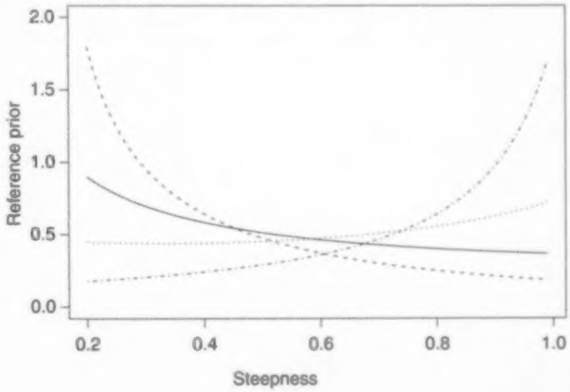
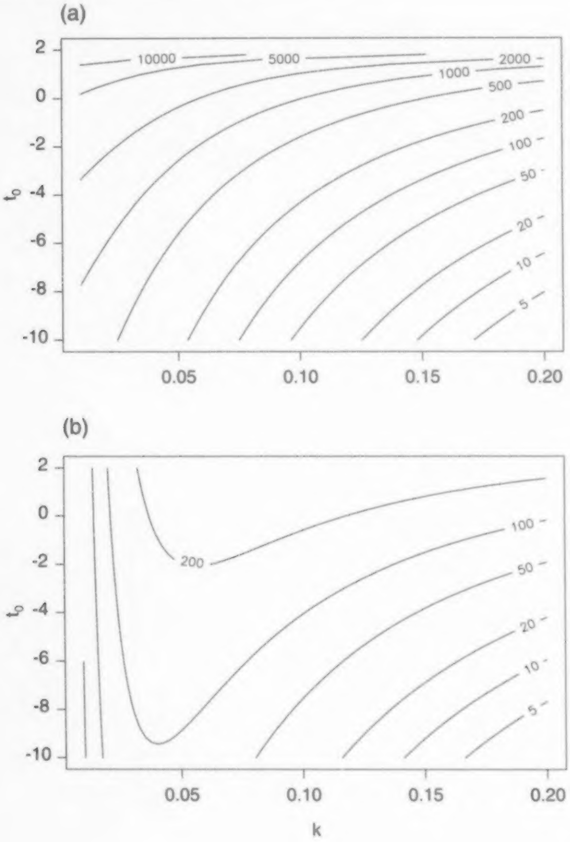


Fig. 4. Joint reference prior for parameters k and t_0 of the von Bertalanffy growth curve, calculated for the age data from p. 136 of Quinn and Deriso (1999). (*a*) Multiplicative lognormal errors and (*b*) additive normal errors.



duction model gives the biomass in year y according to the equation

$$(10) \quad B_y = B_{y-1} + rB_{y-1}\left(1 - \frac{B_{y-1}}{K}\right) - C_{y-1}, \quad r > 0, K > 0$$

where K is carrying capacity and r is the productivity parameter. B_0 is the biomass at the start of the fishery and it is usual to set $B_0 = K$, and this will be assumed here.

It is assumed that a relative biomass index, I_y, measured with multiplicative lognormal error, is observed each year. That is,

$$(11) \quad I_y = QB_y e^{\nu_y}, \quad Q > 0$$

where ν_y are independent and identically distributed $N(0, \sigma^2)$ random errors.

The elements of $I(r, K, Q)$ can not be written in closed form because of the recursive definition of the sequence of biomasses. However, it can be shown (Appendix A) that the resulting Jeffreys' prior can be written in the form

$$\pi(r, K, Q) \propto \frac{1}{Q}\pi(r, K), \quad Q > 0, r > 0, K > 0$$

That is, the Jeffreys' prior for Q, the catchability coefficient, is uniform on the log scale and is independent of the joint Jeffreys' prior for r and K.

Example

The prior density $\pi(r, K)$ was calculated for the catch values of the South Atlantic albacore tuna (*Thunnus alalunga*) used in Polacheck et al. (1993) and Meyer and Millar (1999). It increases with decreasing values of K and r (Fig. 5).

Sequential population analysis

There are many variants of sequential population analysis depending on the nature of the fishery and the data that are available. Here, it will be assumed that instantaneous natural mortality, m, is constant and common to all recruited age classes, and that instantaneous fishing mortality is separable (Quinn and Deriso 1999), that is, can be modeled as a product of annual fishing effort and age-specific selectivity. The data are assumed to be a relative index of numbers-at-age in each year, $I_{a,y}$.

A word of caution is appropriate here. For the model below there are good reasons to have doubts about the appropriateness of the Jeffreys' prior for use as a reasonable reference prior. This issue is taken up in the Discussion.

Let $N_{a,y}$ be the number of age a fish at the start of year y, and let $F_{a,y}$ denote the instantaneous fishing mortality applied to these fish in year y. The separability assumption sets $F_{a,y} = s_a E_y$, where s_a is the vulnerability of age a fish and E_y is the fishing effort in year y. It will be assumed that the fishing efforts are determined external to the sequential population model, that is, the model takes them to be known.

Numbers-at-age are modeled as

$$(12) \quad \begin{aligned} N_{a_0,y} &= R_y, & R_y > 0,\ \ y \ge y_0 \\ N_{a,y_0} &= T_a, & T_a > 0,\ \ a > a_0 \\ N_{a,y} &= N_{a-1,y-1}e^{-(m+F_{a-1,y-1})}, & m \ge 0,\ a > a_0,\ y > y_0 \end{aligned}$$

where R_y is the number of age a_0 recruits at the start of year y, and T_a is the number of age a fish in the start year y_0. The statistical model for the data is

$$(13) \quad I_{a,y} = Q_a N_{a,y} e^{\nu_{a,y}}, \quad Q_a > 0$$

where Q_a is the catchability of age a fish and $\nu_{a,y}$ are independent and identically distributed $N(0, \sigma^2)$ random errors.

If A is the maximum age, then the Jeffreys' prior for the parameter vector

$$\theta = (m, Q_{a_0}, \ldots, Q_A, R_{y_0}, \ldots, R_Y, T_{a_0+1}, \ldots, T_A, s_{a_0}, \ldots, s_A)$$

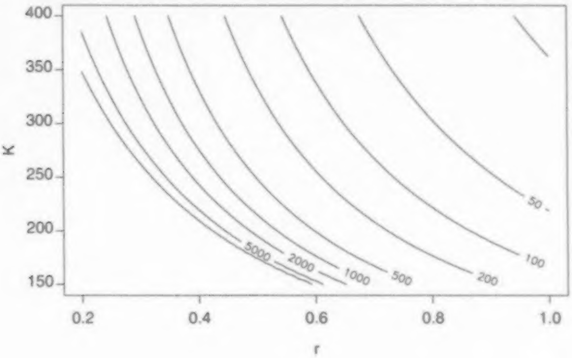
is

$$\pi(\theta) \propto \prod_{a=a_0}^{A} \frac{1}{Q_a} \prod_{y=y_0}^{Y} \frac{1}{R_y} \prod_{a=a_0+1}^{A} \frac{1}{T_a},$$

$$m \ge 0, Q_a > 0, R_y > 0, T_a > 0, s_a \ge 0$$

That is, the Jeffreys' prior for catchabilities, recruitments, and initial numbers in year y_0, are all independent and uni-

Fig. 5. Joint reference prior for parameters K and r of the Schaeffer surplus production model, calculated for the albacore tuna (*Thunnus alalunga*) catch data used in Meyer and Millar (1999).



form on the log scale. The Jeffreys' prior for instantaneous natural mortality and gear vulnerability are also independent and are uniform on $[0, \infty)$. From the transformation of variables formula (1) it follows that the Jeffreys' prior for natural annual survival, $p = e^{-m}$, is $\pi(p) = 1/p$, $0 < p \leq 1$.

The population model used above can be criticized for not making explicit use of catch-at-age data, $C_{a,y}$. Perhaps a more applicable population model would be that of Pope (1972)

$$(14) \quad N_{a,y} = N_{a-1,y-1}e^{-m} - C_{a-1,y-1}e^{-m/2}$$

The Jeffreys' prior under this form would be tedious to calculate because it will involve a large Hessian matrix with elements being summations over terms containing θ, $N_{a,y}$'s and $C_{a,y}$'s. However, it is argued (Discussion) that this may not be a worthwhile pursuit because the Jeffreys' priors, from either eq. 12 or eq. 14, have properties that are questionable in a reference prior.

Discussion

Reference priors have been used in the existing Bayesian fisheries literature, but only in the simplest of cases. These include the use of the "inverse" prior (eq. 3) for standard deviations and for the catchability coefficient Q (i.e., $\pi(Q) = 1/Q$) when the catch data are modeled as lognormal (McAllister et al. 1994; Walters and Ludwig 1994; Punt et al. 1995). More generally, application of Jeffreys' method to lognormal models results in the inverse prior form for all scale parameters, that is, parameters that are additive on the log scale. These include parameter a of the Ricker and Berverton–Holt stock–recruit curves, parameter L_∞ of the von Bertalanffy growth curve, and Q in the surplus production model.

The inverse prior on scale parameters is widely accepted by Bayesian modelers as the appropriate default prior. More generally, however, there is no guarantee that Jeffreys' method, or any other, will produce good reference priors. Indeed, Jeffreys was well aware of the limitations of using formal methods to derive priors and advocated the viewpoint that the reference prior may need to be chosen by consensus when formal methods fail.

The Ricker and Beverton–Holt stock–recruit models and the von Bertalanffy model are all examples of nonlinear regression models. The Jeffreys' prior has previously been used as a reference prior for general nonlinear regression models (e.g., Eaves 1983), and so, with the possible exception of the von Bertalanffy model with additive error, it is reasonable to recommend the Jeffreys' priors derived herein for consideration as reference priors. The exception arises because the L_∞ parameter is a scale parameter in an additive model and (for reasons similar to those justifying the convention to apply Jeffreys' method separately to variance parameters) should be considered separately from the other parameters when applying Jeffreys' method, resulting in a uniform prior for L_∞. The observation error version of surplus production models is also a nonlinear regression model, albeit one where the fitted values are recursively defined (eq. 10), and can reasonably be recommended for consideration as a reference prior.

It is fundamental to note that priors obtained by formal rules depend explicitly on the likelihood function (eq. 2). Thus, in addition to depending on the model formulation and the assumed error structure of the data, such priors can also depend on the values of the explanatory variables. Here, the Jeffreys' prior for the Beverton–Holt stock–recruit curve depends on the values of S_t, for the von Bertalanffy curve it depends on the ages, and for the surplus production model it depends on the catches. That is, the Jeffreys' prior for a parameter is in the context of a given statistical model and experiment.

The explicit dependence of formally derived priors on the likelihood serves as notice to the modeler that specification of the likelihood is conveying information (or misinformation) about the parameters. This can be seen in the sequential population analysis example. The Jeffreys' prior resulted in catchabilities, recruitments, and initial numbers-at-age all being independent and identically distributed as uniform on the log scale. In particular, the prior independence of the recruitments implies that knowing the true values of recruitment in years $y_0,\ldots,Y - 1$ would provide no information at all about the recruitment in year Y. This is not reasonable because knowing the true values of earlier recruitments would at least give some information about the order of magnitude and variability in recruits. Similar criticisms can be made about the catchabilities and initial numbers-at-age. The fault lies with the likelihood—there is nothing in the likelihood conveying the information that the recruitments $R_{y_0},\ldots,R_Y$ are parameters quantifying the *same* biological process over a contiguous sequence of years. It behooves the modeler to include this information, perhaps by modeling recruitments as exchangeable, or as following an autoregressive process.

Agreement on use of reference priors will also need to consider the complexity of deriving and implementing the priors. For many fisheries models, formal methods for deriving priors will be prohibitively challenging. This could be partly overcome by compilation of a catalog of reference priors for fisheries models, so that the modeler could literally pull the prior off the shelf.

Implementation of reference priors could be problematic for some Bayesian software packages. For example, the symbolic BUGS software (Spiegelhalter et al. 1996) requires customized programming to use improper priors and may fail to compile if the prior is too complicated. Complex priors might be more reliably implemented in software that requires explicit algebraic expression of the prior and data likelihood terms.

Fisheries models can be very complicated and of high dimension, and such models are presently well beyond the available statistical methodology for formally deriving potential reference priors. Nonetheless, it would be invaluable to establish general guidelines. These guidelines might, for example, consider the appropriateness of assuming a priori independence of subsets of parameters, thereby enabling a high-dimensional prior to be broken down to more tractable low-dimensional pieces. It would also be worthwhile to examine the sensitivity of the posterior distribution to changes in the prior: if the posterior is insensitive to prior specification, then it might be pragmatic to simplify the specification of reference prior. For example, the Beverton–Holt stock–recruit curve example suggests that a uniform reference prior for h_p, $\rho < h_p < 1$ might be adequate for any reasonable value of S_0. However, if the posterior is very sensitive to the prior, then it becomes important to elicit a good reference prior, or possibly a collection of several reference priors over which the differences in the resulting posteriors can be examined.

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Appendix A

The derivation of the Jeffreys' prior for the Ricker stock–recruit curve model is presented in considerable detail. Only brief detail is provided for the other models.

Ricker stock–recruit curve (*a*, *b*)

Taking the log of eq. 4 gives

$$r_t = \log(R_t) = \log(a) + \log(S_t) - bS_t + \nu_t$$

and the contribution to the log-likelihood from the data value R_t is therefore

$$l_t(a,b,\sigma) = \frac{-1}{2}\log(2\pi\sigma^2) - \frac{(r_t - \log(a) - \log(S_t) + bS_t)^2}{2\sigma^2}$$

Using the a priori independence form of Jeffreys' method, we can remove σ from the likelihood, giving (to within a constant of proportionality)

$$l_t(a,b) = -(r_t - \log(a) - \log(S_t) + bS_t)^2$$

Differentiating gives

$$\frac{\partial l_t}{\partial a} = \frac{2}{a}(r_t - \log(a) - \log(S_t) + bS_t) = \frac{2}{a}\nu_t$$

$$\frac{\partial l_t}{\partial b} = -2S_t(r_t - \log(a) - \log(S_t) + bS_t) = -2S_t\nu_t$$

Hence,

$$\frac{\partial l_t}{\partial a}\frac{\partial l_t}{\partial a} = \frac{4}{a^2}\nu_t^2, \quad \frac{\partial l_t}{\partial b}\frac{\partial l_t}{\partial b} = 4S_t^2\nu_t^2$$

and

$$\frac{\partial l_t}{\partial a}\frac{\partial l_t}{\partial b} = \frac{\partial l_t}{\partial b}\frac{\partial l_t}{\partial a} = \frac{-4S_t}{a}\nu_t^2$$

The expected value of ν_t^2 is σ^2, giving

$$E\left[\frac{\partial l_t}{\partial a}\frac{\partial l_t}{\partial a}\right] = \frac{4}{a^2}\sigma^2, \quad E\left[\frac{\partial l_t}{\partial b}\frac{\partial l_t}{\partial b}\right] = 4S_t^2\sigma^2$$

and

$$E\left[\frac{\partial l_t}{\partial a}\frac{\partial l_t}{\partial b}\right] = E\left[\frac{\partial l_t}{\partial b}\frac{\partial l_t}{\partial a}\right] = \frac{-4S_t}{a}\sigma^2$$

Using the fact that the information matrix for a data set with n independent observations is the sum of the information matrices calculated for each individual observation (Azzalini 1996), we have

$$I(a, b) = 4\sigma^2\begin{pmatrix} \frac{n}{a^2} & -\frac{1}{a}\sum_t S_t \\ -\frac{1}{a}\sum_t S_t & \sum_t S_t^2 \end{pmatrix}$$

and the determinant of this matrix is

$$\det(I(a, b)) = \frac{16\sigma^4}{a^2}\left(n\sum_t S_t^2 - \left[\sum_t S_t\right]^2\right)$$

$$= \frac{16\sigma^4 n}{a^2}\sum_t (S_t - \bar{S})^2$$

where $\bar{S}$ is the average of the S_t, $t = 1,\ldots,n$. Thus, the reference prior for a and b is

$$\pi(a, b) \propto \det(I(a, b))^{1/2} \propto \left(\frac{1}{a^2}\right)^{1/2} = \frac{1}{a}$$

Beverton–Holt stock–recruit curve (*a*, *b*)

Taking the log of eq. 5 gives

$$r_t = \log(R_t) = \log(a) + \log(S_t) - \log(1 + bS_t) + \nu_t$$

and the relevant contribution to the log-likelihood from the data value R_t is therefore

$$l_t(a, b) = -(r_t - \log(a) - \log(S_t) + \log(1 + bS_t))^2$$

Differentiation gives

$$\frac{\partial l_t}{\partial a} = \frac{2}{a}(r_t - \log(a) - \log(S_t) + \log(1 + bS_t)) = \frac{2}{a}\nu_t$$

$$\frac{\partial l_t}{\partial b} = -\frac{2S_t}{1 + bS_t}(r_t - \log(a) - \log(S_t) + \log(1 + bS_t)) = -\frac{2S_t}{1 + bS_t}\nu_t$$

Hence,

$$I(a,b) = 4\sigma^2 \begin{pmatrix} \dfrac{n}{a^2} & -\dfrac{1}{a}\sum_t \dfrac{S_t}{1+bS_t} \\ -\dfrac{1}{a}\sum_t \dfrac{S_t}{1+bS_t} & \sum_t \left(\dfrac{S_t}{1+bS_t}\right)^2 \end{pmatrix}$$

Denoting $y_t(b) = S_t/(1 + bS_t)$, the determinant of $I(a, b)$ is

$$\begin{aligned} \det(I(a,b)) &= \frac{16\sigma^2}{a^4}\left(n\sum_t y_t^2(b) - \left[\sum_t y_t(b)\right]^2\right) \\ &= \frac{16\sigma^4 n}{a^2}\sum_t (y_t(b) - \bar{y}(b))^2 \\ &= \frac{16\sigma^4 n(n-1)}{a^2}\,\frac{\sum_t (y_t(b) - \bar{y}(b))^2}{n-1} \\ &= \frac{16\sigma^4 n(n-1)}{a^2}\,\mathrm{var}(y(b)) \end{aligned}$$

where $\bar{y}(b)$ is the average of $y_t(b)$, $t = 1,\ldots,n$ and $\mathrm{var}(y(b))$ is their sample variance. Thus, the reference prior for a and b is

$$\pi(a,b) \propto \det(I(a,b))^{1/2}) \propto \frac{\mathrm{sd}(y(b))}{a}$$

where $\mathrm{sd}(y(b))$ is the sample standard deviation of $y_t(b)$, $t = 1,\ldots,n$.

Remarks

The reference prior $\pi(b) = \mathrm{sd}(y(b))$ is proper, that is, is integrable with respect to b. This is established by using the following two inequalities:

(*i*) $\mathrm{sd}(y(b)) \le \mathrm{sd}(S)$, and

(*ii*) $\mathrm{sd}(y(b)) \le \dfrac{c}{b^2}$ for some constant c.

The first inequality is established by virtue of the fact that, for any i and j and all $b > 0$, $|y_i(b) - y_j(b)| < |S_i - S_j|$. The second inequality is established by noting that

$$\mathrm{sd}(y(b)) = \frac{1}{b}\,\mathrm{sd}\left(\frac{1}{1+bS}\right)$$

To obtain the prior on the steepness parameter, write eq. 7 as

$$h_\rho = 1 - \frac{1-\rho}{1+\rho b S_0}$$

The inverse of this is

$$b = \frac{h_\rho - \rho}{\rho S_0(1-h_\rho)}$$

which has the derivative

$$\frac{\partial b}{\partial h_\rho} = \frac{1-\rho}{\rho S_0(1-h_\rho)^2}$$

von Bertalanffy growth curve (L_∞, k, t_0)

Letting a_i denote the age of the ith fish in the sample, taking the log of eq. 8 gives

$$\log(L_{a_i}) = \log(L_\infty) + \log(1 - e^{-k(a_i - t_0)}) + \nu_i$$

and the relevant contribution to the log-likelihood from the data value L_{a_i} is therefore

$$l_i(L_\infty, k, t_0) = -(\log(L_{a_i}) - \log(L_\infty) - \log(1 - e^{-k(a_i - t_0)}))^2$$

Differentiating gives

$$\frac{\partial l_{a_i}}{\partial L_\infty} = \frac{2}{L_\infty} \nu_i$$

$$\frac{\partial l_{a_i}}{\partial k} = \frac{2(a_i - t_0)e^{-k(a_i - t_0)}}{1 - e^{-k(a_i - t_0)}} \nu_i$$

$$\frac{\partial l_{a_i}}{\partial t_0} = -\frac{2k e^{-k(a_i - t_0)}}{1 - e^{-k(a_i - t_0)}} \nu_i$$

Hence,

$$I(L_\infty, k, t_0) = 4\sigma^2 \begin{pmatrix} \frac{n}{L_\infty^2} & \frac{1}{L_\infty}\sum_i \frac{\delta_i e^{-k\delta_i}}{1 - e^{-k\delta_i}} & -\frac{k}{L_\infty}\sum_i \frac{e^{-k\delta_i}}{1 - e^{-k\delta_i}} \\ \frac{1}{L_\infty}\sum_i \frac{\delta_i e^{-k\delta_i}}{1 - e^{-k\delta_i}} & \sum_i \left[\frac{\delta_i e^{-k\delta_i}}{1 - e^{-k\delta_i}}\right]^2 & -k\sum_i \delta_i \left[\frac{e^{-k\delta_i}}{1 - e^{-k\delta_i}}\right]^2 \\ -\frac{k}{L_\infty}\sum_i \frac{e^{-k\delta_i}}{1 - e^{-k\delta_i}} & -k\sum_i \delta_i \left[\frac{e^{-k\delta_i}}{1 - e^{-k\delta_i}}\right]^2 & k^2\sum_i \left[\frac{e^{-k\delta_i}}{1 - e^{-k\delta_i}}\right]^2 \end{pmatrix}$$

where $\delta_i = a_i - t_0$.

The determinant of $I(L_\infty, k, t_0)$ can be calculated by many familiar software packages, or directly by using the formula for the determinant of three-by-three matrices (e.g., p. 150 of Jacob 1990). From this formula it can be noted that L_∞ appears in the determinant only as a multiplicative constant.

Schaefer surplus production model (Q, K, r)

Taking the log of eq. 11 gives

$$i_y = \log(I_y) = \log(Q) + \log(B_y) + \nu_y$$

The relevant contribution to the log-likelihood from the data value I_y is therefore

$$l_y = -(i_y - \log(Q) - \log(B_y))^2$$

Letting $k = 1/K$ and differentiating gives

$$\frac{\partial l_y}{\partial Q} = \frac{2}{Q} \nu_y$$

$$\frac{\partial l_y}{\partial r} = \frac{\partial l_y}{\partial B_y} \frac{\partial B_y}{\partial r} = \frac{2}{B_y} \frac{\partial B_y}{\partial r} \nu_y$$

$$\frac{\partial l_y}{\partial K} = \frac{\partial l_y}{\partial k} \frac{\partial k}{\partial K} = \frac{\partial l_y}{\partial B_y} \frac{\partial B_y}{\partial k} \frac{(-1)}{K^2} = \frac{-2}{K^2 B_y} \frac{\partial B_y}{\partial k} \nu_y$$

The above derivatives of B_y are obtained from the recursive surplus production eq. 10, which can be denoted $B_y = g(B_{y-1}, r, K)$. Then,

$$\frac{\partial B_y}{\partial r} = \frac{\partial g(B_{y-1}, r, K)}{\partial r} = \frac{\partial g(B, r, K)}{\partial B} \frac{\partial B}{\partial r} /_{B_{y-1}, r, K} + \frac{\partial g(B, r, K)}{\partial r} /_{B_{y-1}, r, k}$$

and similarly for $\frac{\partial B_y}{\partial k}$. The partial derivatives of g are

$$\frac{\partial g(B, r, K)}{\partial B} = 1 + r - 2rkB$$

$$\frac{\partial g(B, r, K)}{\partial r} = B(1 - kB)$$

$$\frac{\partial g(B, r, K)}{\partial k} = -rB^2$$

Sequential population analysis (*m*, *Q*, *R*, *T*, *s*)

Taking the log of eq. 13 gives

$$\log(I_{a,y}) = \log(Q_a) + n_{a,y} + \nu_{a,y}$$

where $n_{a,y} = \log(N_{a,y})$. The relevant contribution to the log-likelihood from the data value $I_{a,y}$ is therefore

$$l_{a,y} = -(\log(I_{a,y}) - \log(Q_a) - n_{a,y})^2$$

On the log scale, numbers-at-age can be written as

$$\begin{aligned} n_{a,y} &= n_{a-1,y-1} - m - F_{a-1,y-1} \\ &= \begin{cases} n_{a_0,y-(a-a_0)} - (a-a_0)m - \sum_{i=1}^{a-a_0} F_{a-i,y-i}, & y - y_0 \geq a - a_0 \\ n_{a-(y-y_0),y_0} - (y-y_0)m - \sum_{i=1}^{y-y_0} F_{a-i,y-i}, & \text{otherwise} \end{cases} \\ &= \begin{cases} \log(R_{y-(a-a_0)}) - (a-a_0)m - \sum_{i=1}^{a-a_0} s_{a-i}E_{y-i}, & y - y_0 \geq a - a_0 \\ \log(T_{a-(y-y_0)}) - (y-y_0)m - \sum_{i=1}^{y-y_0} s_{a-i}E_{y-i}, & \text{otherwise} \end{cases} \end{aligned}$$

Differentiation of the log-likelihood with respect to the model parameters thus gives

$$\frac{\partial l_{a,y}}{\partial m} = \begin{cases} -2(a-a_0)\nu_{a,y}, & y \geq y_0 + (a - a_0) \\ -2(y-y_0)\nu_{a,y}, & \text{otherwise} \end{cases}$$

$$\frac{\partial l_{a,y}}{\partial Q_\alpha} = \begin{cases} \frac{2}{Q_\alpha}\nu_{\alpha,y}, & a = \alpha \\ 0, & \text{otherwise} \end{cases}$$

$$\frac{\partial l_{a,y}}{\partial R_\xi} = \begin{cases} \frac{2}{R_\xi}\nu_{a,y}, & y = \xi + a - a_0 \\ 0, & \text{otherwise} \end{cases}$$

$$\frac{\partial l_{a,y}}{\partial T_\alpha} = \begin{cases} \frac{2}{T_\alpha}\nu_{a,y}, & y = y_0 + a - \alpha \\ 0, & \text{otherwise} \end{cases}$$

$$\frac{\partial l_{a,y}}{\partial s_\alpha} = \begin{cases} E_{y-(a-\alpha)}\nu_{a,y}, & y \geq y_0 + (a - \alpha) > y_0 \\ 0, & \text{otherwise} \end{cases}$$

It follows that

$$\det(I(\theta))^{1/2} \propto \left(\prod_{a=a_0}^{A} \frac{1}{Q_a^2} \prod_{y=y_0}^{Y} \frac{1}{R_y^2} \prod_{a=a_0+1}^{A} \frac{1}{T_a^2} \right)^{1/2} = \prod_{a=a_0}^{A} \frac{1}{Q_a} \prod_{y=y_0}^{Y} \frac{1}{R_y} \prod_{a=a_0+1}^{A} \frac{1}{T_a}$$

A regional meta-model for stock–recruitment analysis using an empirical Bayesian approach

D.G. Chen and L. Blair Holtby

Abstract: A regional stock–recruitment meta-model is developed using a hierarchical Bayesian framework to combine information from multiple fish populations. The use of the meta-model is illustrated through analysis of the regional stock–recruitment parameters of the coho salmon (*Oncorhynchus kisutch*) within two large fisheries management units in southern and northern British Columbia. We construct our regional prior distribution from an analysis of all stock–recruitment data rather than by the more usual approach of assuming a prior distribution. That preliminary analysis indicated that the regional prior distribution for the two parameters of the Ricker model was bivariate normal–lognormal (NLN) with a high degree of correlation between the two Ricker parameters. Because this distribution had not been fully developed, we formulated the density function for the NLN distribution and proved some of its important properties. An empirical Bayesian approach was then used to estimate the regional distributions of the Ricker parameters and derived management parameters. Characterization of the distributional properties of productivity within management regions is a necessary step for resource managers seeking to prosecute mixed-stock fisheries while conserving population diversity.

Résumé : Nous avons mis au point un métamodèle de stock–recrutement régional à l'aide d'un cadre hiérarchique Bayésien dans le but de combiner les renseignements provenant de plusieurs populations de poissons. Nous proposons en exemple de notre méta-modèle une analyse des paramètres régionaux de stock–recrutement de saumons coho (*Oncorhynchus kisutch*) provenant de deux grandes unités de gestion de la pêche dans le sud et le nord de la Colombie-Britannique. Nous avons basé la distribution régionale a priori sur une analyse de toutes les données de stock–recrutement au lieu de simplement assumer une distribution a priori comme on le fait couramment. Cette analyse préliminaire a révélé que la distribution régionale a priori des deux paramètres du modèle de Ricker est de type normal–lognormal (NLN) à deux variables avec un fort degré de corrélation entre les deux paramètres. Parce que la distribution n'avait pas été complètement décrite, nous avons calculé la fonction de densité de la distribution NLN et mis en lumière certaines de ses propriétés importantes. Une méthode empirique Bayésienne nous a ensuite permis d'estimer les distributions régionales des paramètres de Ricker et d'obtenir les paramètres de gestion. La caractérisation des propriétés de la distribution de la productivité dans une unité de gestion est une opération essentielle lorsque les gestionnaires d'une ressource cherchent à maintenir l'exploitation d'une pêche commerciale comprenant plusieurs stocks tout en conservant la diversité de la population.

[Traduit par la Rédaction]

Introduction

The management of mixed-stock fisheries has proven to be one of the most persistent and difficult problems facing resource managers. Such problems are most severe in anadromous species where fisheries occur in the ocean on numerous populations of differing productivities. Mixed-stock fisheries have been implicated in regional declines in most anadromous species, including all of the Pacific salmon (*Oncorhynchus* sp.), Atlantic salmon (*Salmo salar*), striped bass (*Morone saxatilis*), the river herrings (*Alosa pseudoharengus* and *A. aestivalis*), and shad (*Alosa sapidissima*). The threat to the stock diversity of Pacific salmon that such fisheries pose has long been recognized (Paulik et al. 1967; Ricker 1973; McDonald 1981). Nevertheless, despite extensive study of the problems posed by such fisheries and approaches to achieve optimal harvest while protecting stock diversity (Ricker 1958; Collie et al. 1990; Kope 1992), the failure to recognize and, more importantly, react appropriately to severe overfishing in intense and highly mixed-stock troll fisheries precipitated unprecedented closures of ocean coho salmon (*O. kisutch*) fisheries in Pacific Canada beginning in 1998 that remain in place.

Of the Pacific salmon, coho salmon are particularly susceptible to the problems of mixed-stock fisheries. Coho generally remain close to shore throughout their approximately 18-month life in the ocean and recruit to fisheries during their second summer in the ocean (Sandercock 1991). Feeding coho readily pursue moving lures, making them highly vulnerable to commercial and recreational troll fisheries. Coho salmon from many populations mingle in feeding areas (e.g., Argue et al. 1983; Pacific Salmon Commission 1994) and an ocean fishery can capture coho from nearly every population over a surprisingly large area. For

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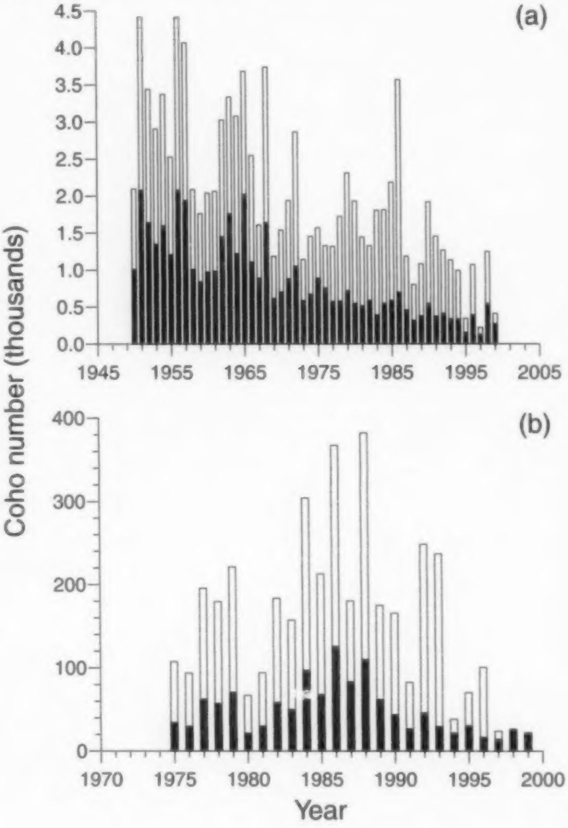
example, the intense troll fisheries off the west coast of Vancouver Island captured significant proportions (>25%) of the fish captured from nearly all coho populations in southern British Columbia (all streams draining the west coast of Vancouver Island and all streams draining into the Strait of Georgia and its inlets, including the Fraser River and its tributaries) and Washington (WA; all of the WA coast north of the Columbia River, including the Strait of Juan de Fuca and Puget Sound) (Pacific Salmon Commission 1994). Exploitation rates on coho salmon in ocean fisheries regularly exceeded 70% prior to stock collapses in the mid-1990s (e.g., Holtby et al. 1999; Irvine et al. 2000). The severity of the population declines within subregions was directly attributed to mesoscale differences in productivity (Holtby et al. 1999). Such subregional patterning of population decline is readily distinguished from the widespread declines in abundance that have occurred in the southern portions of coho range (e.g., Brown et al.1994; Weitkamp et al. 1995; Beamish et al. 1999), which have generally been attributed to declines in the survival of juveniles entering the ocean caused by changes in ocean climate (Coronado and Hilborn 1998; Beamish et al. 2000) or to freshwater habitat loss and damage (e.g., Beechie et al. 1994; Bradford and Irvine 2000). The large declines in abundance in southern and northern coastal British Columbia (B.C.; Fig. 1) in the mid-1990s prompted the Canadian Department of Fisheries and Oceans, which is responsible for the management of anadromous species such as the Pacific salmon, to implement several policy and management initiatives aimed at conserving and rebuilding coho salmon populations. One of the policy initiatives was the development of a Wild Salmon Policy (WSP) (http://www-comm.pac.dfo-mpo.gc.ca/wsp-sep-consult/wsp/wsp.pdf). The WSP is structured around general principles derived from the precepts of conservation biology and precautionary management (DFO 2000). One of those principles requires that the many hundreds of coho populations will be aggregated, for management purposes, into a small number of conservation or management units (MU). The grouping of populations would be based on genetic and phenotypic similarities among the component populations but would also require that the component populations have similar fisheries profiles so that they are manageable as aggregates in mixed-stock fisheries.

Another principle of the WSP stipulates that a target and limit reference point would be established for each MU. Although it may be straightforward to determine such reference points for single populations, determining the reference points for a MU is more involved. When the fish are caught in mixed-stock fisheries, as is the case in coho salmon, any exploitation rate is unlikely to be optimal for all of the component populations. This is the classic mixed-stock fisheries problem. Setting the exploitation rate in such a situation would then become a public policy decision balancing diversity and harvest. To form such a policy decision, it is necessary both to describe the distribution of productivities within each MU and to model the consequences of policy options on the status of component populations within the MU.

Fig. 1. Time series of coho salmon (*Oncorhynchus kisutch*) escapement (solid bars) and catch (open bars) in (*a*) northern British Columbia (NBC) and (*b*) southern British Columbia (SBC). The values are an average stream index (see Holtby et al. 1999) for NBC and estimates of total escapement and catch for a subset of Thompson River populations for SBC.



In this paper, we describe the development of an empirical Bayesian approach for estimating the regional distributions of the two parameters of the Ricker stock–recruitment function and some associated management parameters (Hilborn and Walters 1992). We formally develop a new probability distribution (bivariate normal–lognormal or NLN) to accommodate both the different marginal distributions for α and β and their correlation structure. We incorporate the NLN distribution into a hierarchical Bayesian model for estimation of the regional distribution of α and β. Our approach is empirical because we use sample data to estimate the unknown regional parameters in the NLN prior distribution rather than assuming a known prior distribution as would be done in a standard Bayesian analysis.

Preliminary analysis for regional distributions

For Pacific salmon, stock–recruitment (S–R) analysis models the functional relationship between the escapement (S_t, i.e., the number of spawners) and the number of recruits that they produce (R_t) that are alive at some specified time (t), typically either immediately before the fishery, as is the case here, or at the inception of spawning. Coho salmon recruit to fisheries in their second summer in the ocean, and all fish that survive the fishery return to their natal streams to spawn in the fall of the same year. We assume that there is negligible natural mortality after the fishery begins and that recruitment is therefore the sum of escapement and catch. This is a conservative assumption for production modeling because it will underestimate recruitment and thus productivity.

Time series of coho escapement indices from populations within the Thompson River and its tributaries in southern British Columbia (SBC) (Fig. 2) and from populations within Statistical Area 6 in northern British Columbia (NBC) were used for this regional meta-model development. Escapement data from over 100 populations in NBC for the years 1950 to 1999 and 60 populations in SBC for the years 1975 to 1999 were available. Details of the data preparation are found in Holtby et al. (1999) for NBC and in Irvine et al. (2000) for SBC. Escapement estimates in SBC streams were corrected on a river-specific basis for observer efficiencies (Irvine et al. 2000), but no correction was possible in the NBC streams. No relationship between the correction factor and the size of the system was detected in the SBC systems. Stock-specific catch was estimated using estimates of the exploitation rate. We assumed that the annual exploitation rate was equal for all populations within each of the two regions. For NBC systems, the exploitation rate was derived from recoveries of coded-wire-tagged fish in ocean fisheries released from indicator stocks in the Skeena River from 1988 onward (Holtby et al. 1999). For the remaining years, the exploitation rate was estimated from records of total fishery effort in the ocean fisheries of northern British Columbia and southeast Alaska and empirical effort – exploitation rate relationships (Pacific Salmon Commission 2002). In SBC, exploitation rates were estimated from coded-wire-tagged releases from Thompson River and other southern British Columbian hatcheries (1975–1997) and direct estimates of the numbers of Thompson River fish in ocean fisheries (1998, 1999; Irvine et al. 2000).

In both areas and particularly in NBC, the data series were fragmentary in many systems. In NBC where there are two freshwater ages and adults return over 2 years from each brood year, the data for each population consisted of triplets of values where escapement had been indexed in a brood year and in both return years. In SBC, there is one freshwater age and all spawners return in the same year. Therefore, in SBC, the data consisted of pairs of values where escapement had been indexed in the brood year and the single return year. In both NBC and SBC, the escapement counts were treated as indices of escapement and not as absolute estimates of escapement. These data sets, and in particular the one from NBC, initially included all streams that had ever been enumerated, even if for only 1 year. Short periods of observation were common at the beginning of data collection. This suggested to us that those making the escapement estimates felt that estimates were particularly unreliable in those systems. The minimum standard for inclusion (five stock–recruitment observations) was developed in consultation with field staff familiar with these data. Stocks with five or fewer stock–recruitment observations were discarded, leaving 83 populations in NBC and 50 in SBC.

The most commonly used *S–R* models are the Ricker (1975) and the Beverton and Holt (1957) models. The Ricker model with a lognormal error term is used in this paper:

$$R_t = S_t \exp(\alpha - \beta S_t)\exp(\varepsilon_t)$$

where α is the parameter measuring reproductive performance at low stock size with $\exp(\alpha)$ being the maximum recruits per spawner and β is the parameter representing density dependence in juvenile survival rate; ε_t is a normally distributed process error with mean 0 and standard deviation σ. This model can be linearized as

$$(1) \qquad y_t = \log(R_t/S_t) = \alpha - \beta S_t + \varepsilon_t$$

whereupon the parameters α and β can be estimated by simple least-squares regression. Having estimates of α and β, fishery management parameters, such as S_{MSY} (the optimal stock size at maximum sustainable yield (MSY)) and μ_{MSY} (exploitation rate), can be calculated based on the formulations from Hilborn (1985), Hilborn and Walters (1992), and Quinn and Deriso (1999).

Barrowman and Myers (2000) suggested that the Ricker and Beverton–Holt models poorly fit the stock–recruitment data for coho salmon. Their assertion was justified for the Beverton–Holt model, and in our preliminary tests, the Beverton–Holt model did give unrealistically high estimates of the slope at the origin (α) and was not considered further. In the case of the Ricker model, support for their assertion (Barrowman and Myers 2000, their fig. 2) was, in our opinion, tenuous. Our experience is similar to that of Myers et al. (1999), who concluded that the Ricker model usually provides reasonable estimates of α for management purposes. We briefly examined some three-parameter *S–R* models. The results of those analyses did suggest that there was some nonlinearity in the Ricker fits. However, examination of many of the individual fits showed no consistent patterning in the residuals, and we concluded that the Ricker model was adequate for these data. Other *S–R* functions (e.g., the hockey stick; Barrowman and Myers 2000) were not considered, although our framework could be readily extended to other *S–R* functions.

When extended to a region, the Ricker model (eq. 1) becomes $y_{it} = \ln(R_{it}/S_{it}) = \alpha_i - \beta_i S_{it} + \varepsilon_{it}$, where $\varepsilon \sim N(0,\sigma_i^2)$ and $t = 1$ to n_i (dependent on population i), and $i = 1$ to 83 for NBC and $i = 1$ to 50 for SBC. The Ricker model (eq. 1) was fit to each population independently (Figs. 3*a*, 3*b*). For NBC coho, the Ricker α_i are centrally distributed around 1.5 (Figs. 3*a*, 4*a*) and the Ricker β_i is highly skewed toward lower values (Fig. 4*b*). The distributions of α_i and β_i are similar in SBC (Figs. 3*b*, 4*e*, 4*f*), although the average values of both parameters are greater in SBC. In both NBC and SBC, both α_i and $\ln(\beta_i)$ are normally distributed (Figs. 4*d*, 4*h*). This is confirmed by the Kolmogorov–Smirnov distribution test (Table 1).

Regional meta-model

Our primary interest is in the regional populations from which the 83 and 50 populations (NBC and SBC, respectively) were drawn rather than in the individual populations. Independently fitting the Ricker model to the individual population results in a set of parameters estimates α_i and β_i

Fig. 2. Geographical locations of the two management regions of Area 6 (northern British Columbia) and the Thompson River and tributaries (southern British Columbia) on the west coast of Canada.

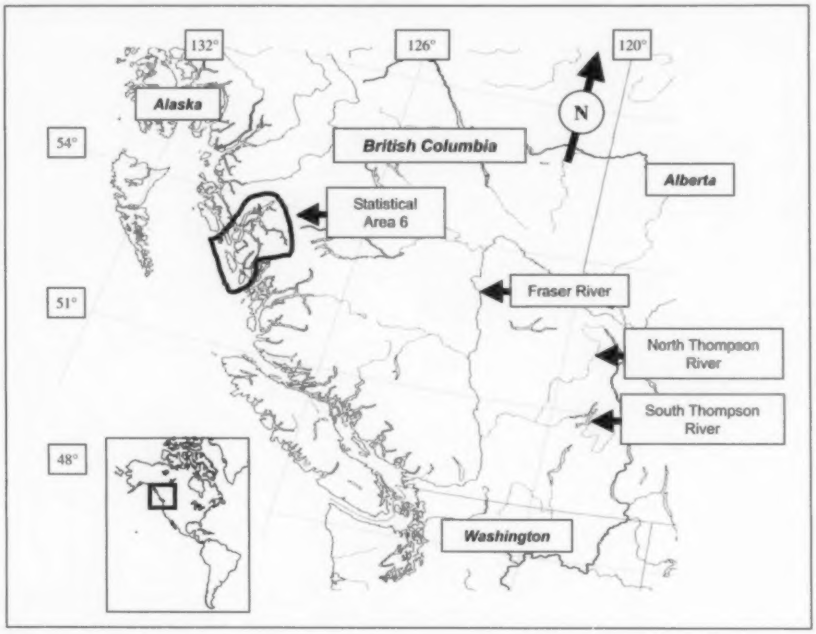
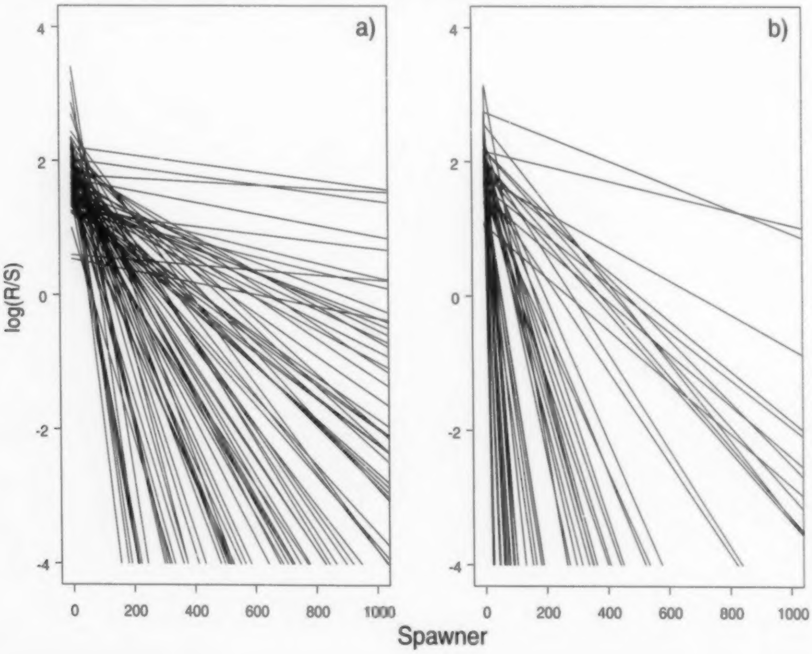

132°
126°
120°
Alaska
N
British Columbia
54°
Alberta
Statistical Area 6
51°
Fraser River
North Thompson River
South Thompson River
48°
Washington

Fig. 3. The Ricker stock–recruitment model fit to the substocks in the management region: (*a*) northern British Columbia (NBC); (*b*) southern British Columbia (SBC). Each line represents the model fit to an individual coho population.



within-sample estimates of $\sigma^2_{\alpha_i}$ and $\sigma^2_{\beta_i}$ for each of the two regions but does not provide a basis for inference about the two regional populations. These estimates are presumably drawn from regional populations with regional means α_R and β_R and among-stock true variances σ^2_{α} and σ^2_{β}. Furthermore, these regional Ricker parameters α_i and β_i should be correlated with the regional coefficient ρ. The process of estimating the regional parameters can be accomplished with

Fig. 4. (*a*–*d*) Northern British Columbia management region: (*a* and *b*) histograms for the Ricker parameters α_i and β_i, respectively; (*c*) sample correlation coefficient of α_i and β_i in the region for each of the 83 populations; (*d*) histogram for the log Ricker density-dependent parameter β_i to demonstrate the fit of a lognormal distribution. (*e*–*h*) The corresponding plots for the southern British Columbia management region. The number of populations summarized in the density histograms (*N*) is indicated for northern and southern British Columbia (83 and 50, respectively).

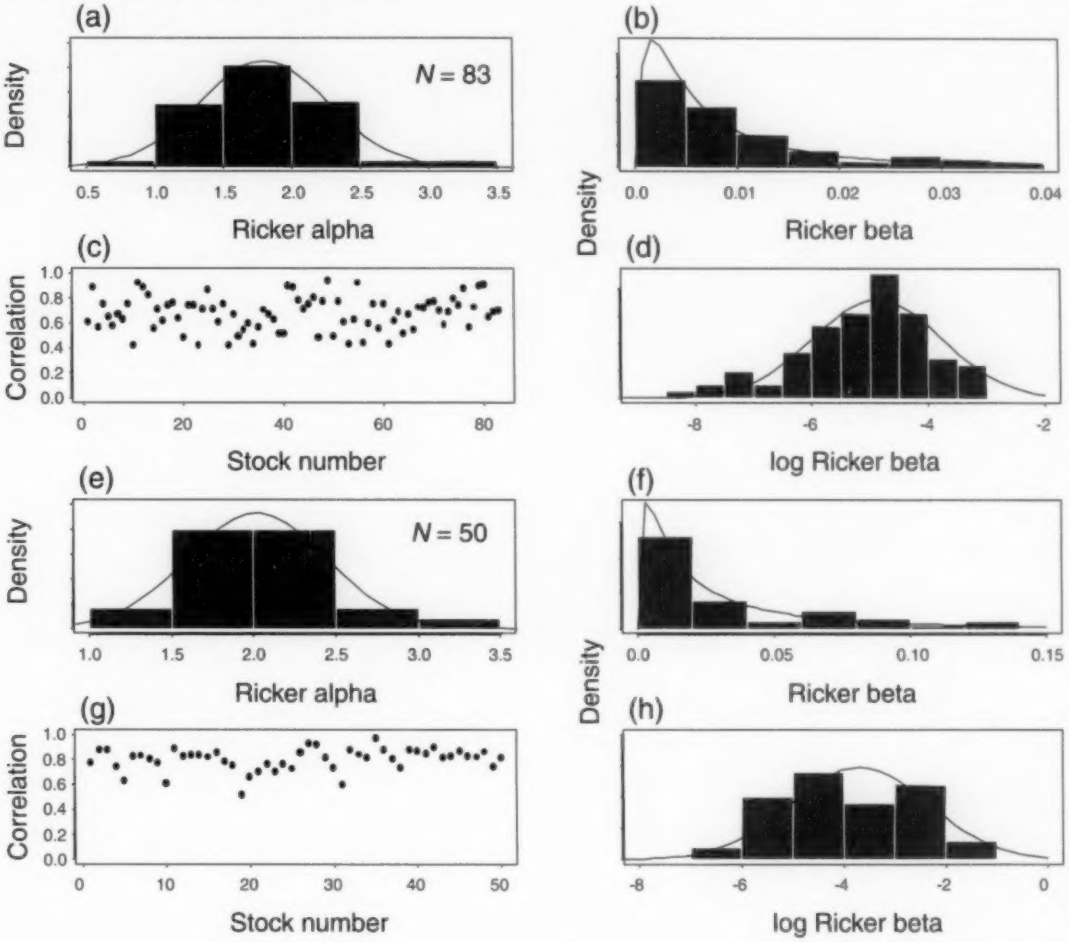


Table 1. Kolmogorov–Smirnov (KS) test statistics for the distributions of the Ricker parameters α_i and β_i corresponding to Fig. 4 for northern British Columbia (NBC) and southern British Columbia (SBC).

Region	Ricker	Distribution	KS value	*p* value
NBC	α	Normal	0.076	$p > 0.250$
	β	Normal	0.179	$p < 0.001$
		Lognormal	0.078	$p > 0.223$
SBC	α	Normal	0.1095	$p > 0.134$
	β	Normal	0.2544	$p < 0.001$
		Lognormal	0.0735	$p > 0.704$

a two-stage hierarchical Bayesian model where stage 1 consists of fitting a Ricker model (1) with lognormally distributed errors to each individual population $i = 1$ to K (83 for NBC and 50 for in SBC) and stage 2 estimates the regional Ricker parameters α_i and β_i that are distributed as a bivariate NLN distribution.

In the analysis of individual populations, we found that the regional prior distribution is in fact a NLN distribution. Therefore the regional meta-model can then be formulated as

$$(2)\quad \begin{cases} \text{Ricker model for each stream } i\text{:} \\ \qquad y_{it} = \ln\left(\dfrac{R_{it}}{S_{it}}\right) = \alpha_i - \beta_i S_{it} + \varepsilon_{it} \\ \qquad \varepsilon_{it} \sim N(0, \sigma_i^2);\ i = 1,\ldots,K;\ t = 1,\ldots,n_i \\ \text{Regional prior distribution:} \\ \qquad (\alpha_i, \beta_i) \sim \mathrm{NLN}(\alpha_R, \sigma_\alpha^2, \beta_R, \sigma_\beta^2, \rho) \end{cases}$$

We present a graphical description of this two-stage hierarchical model (Fig. 5). In model 2 (eq. 2), NLN(α_i, β_i; α_R, σ_α^2, β_R, σ_β^2, ρ) denotes the joint bivariate NLN distribution.

Because this distribution is not formally formulated, we propose and prove (Appendix A) the NLN with a density function defined as

$$(3)\quad \mathrm{dNLN}(\alpha_i,\beta_i;\alpha_R,\sigma_\alpha^2,\beta_R,\sigma_B^2,\rho) = \frac{1}{2\pi\sigma_\alpha\sigma_\beta\sqrt{1-\rho_\tau^2}\beta_i}\exp\left\{-\frac{1}{2(1-\rho_\tau^2)}\left[\left(\frac{\alpha_i-\alpha_R}{\sigma_\alpha}\right)^2 - 2\rho_\tau\frac{\alpha_i-\alpha_R}{\sigma_\alpha}\frac{\ln\beta_i-\beta_R}{\sigma_\beta}+\left(\frac{\ln\beta_i-\beta_R}{\sigma_\beta}\right)^2\right]\right\}$$

where α_R and σ_α^2 are the mean and variance parameters for α_i; β_R and σ_β^2 are the mean and variance for $\ln(\beta_i)$; ρ is the correlation coefficient for α_i and β_i; and

$$\rho_\tau = \frac{\rho\sqrt{\exp(\sigma_\beta^2)-1}}{\sigma_\beta}.$$

The notation dNLN denotes the density function for the NLN distribution. A similar notational shorthand where "d" is prefixed to the name of the distribution has been used elsewhere in this paper, for example, "dNorm" denotes the density function of the normal distribution.

The posterior distribution of α_i and β_i conditional on the *S–R* data and the regional prior distribution (eq. 3) would then be

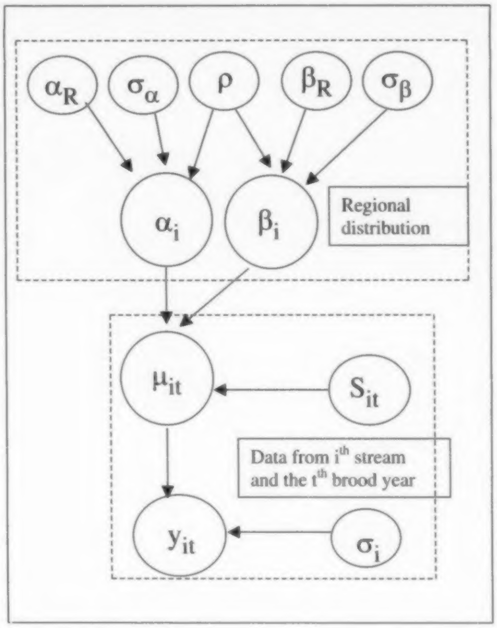
$$(4)\quad \alpha_i,\beta_i|S_{it},y_{it},\alpha_R,\sigma_\alpha^2,\beta_R,\sigma_\beta^2,\rho \propto \mathrm{dNorm}(y_{it};\alpha_i-\beta_iS_{it},\sigma_i^2)\times\mathrm{dNLN}(\alpha_i,\beta_i;\alpha_R,\sigma_\alpha^2,\beta_R,\sigma_\beta^2,\rho)$$

This posterior distribution (eq. 4) could also be used to provide corrected estimates of the (α_i, β_i) pairs using the regional information to provide a more biologically reasonable value for each stock by combining information from both the region and the stock itself (Myers and Mertz 1998).

We have shown that the conditional distribution of $\alpha_i|\beta_i$ is also normally distributed with mean $\alpha_R + \rho_\tau\frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i - \beta_R)$ and variance $\sigma_\alpha^2(1 - \rho_\tau^2)$ (see Appendix A). With simple mathematical manipulations, we can prove that

$$\begin{aligned}
(5)\quad & \alpha_i,\beta_i|S_{it},y_{it},\alpha_R,\sigma_\alpha^2,\beta_R,\sigma_\beta^2,\rho \\
&\propto \mathrm{dNorm}(y_{it};\alpha_i-\beta_iS_{it},\sigma_i^2)\times\mathrm{dNLN}(\alpha_i,\beta_i;\alpha_R,\sigma_\alpha^2,\beta_R,\sigma_\beta^2,\rho) \\
&= \mathrm{dNorm}(y_{it};\alpha_i-\beta_iS_{it},\sigma_i^2) \times \mathrm{dNorm}\left(\alpha_i,\alpha_R+\rho_\tau\frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i-\beta_R),\sigma_\alpha^2(1-\rho_\tau^2)\right) \times \mathrm{dLNorm}(\beta_i;\beta_R,\sigma_\beta^2) \\
&= \mathrm{dNorm}\left(y_{it}+\beta_iS_{it},\alpha_R+\rho_\tau\frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i-\beta_R),\sigma_\alpha^2(1-\rho_\tau^2)+\sigma_i^2\right) \\
&\times\mathrm{dNorm}\left(\alpha_i,\frac{y_{it}+\beta_iS_{it}}{\sigma_i^2}+\frac{\alpha_R+\rho_\tau\frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i-\beta_R)}{\sigma_\alpha^2(1-\rho_\tau^2)},\frac{1}{\sigma_i^2}+\frac{1}{\sigma_\alpha^2(1-\rho_\tau^2)}\right)\mathrm{dLNorm}(\beta_i;\beta_R,\sigma_\beta^2)
\end{aligned}$$

Fig. 5. Graphical illustration of the regional meta-model.



Empirical Bayesian estimation

The dependence of the posterior distribution (eq. 5) on five unknown regional parameters (α_R, σ_α^2, β_R, σ_β^2, ρ) effectively precludes the implementation of a fully Bayesian hierarchical approach. However, an empirical Bayesian approach can be developed in which the observed *S–R* data are used to estimate the distribution of the regional parameters (Carlin and Louis 1998).

Because the primary objective is to obtain the regional distribution, the *S–R* parameters α_i and β_i are nuisance parameters; therefore, these parameters can be integrated out with the marginal likelihood function to estimate the regional parameters (α_R, σ_α^2, β_R, σ_β^2, ρ):

$$(6)\quad L(\alpha_R, \sigma_\alpha^2, \beta_R, \sigma_\beta^2, \rho) = \prod_{i=1}^{K}\prod_{t=1}^{n_i}\int_{\alpha_i}\int_{\beta_i} \mathrm{dNorm}(y_{it}; \alpha_i - \beta_i S_{it}, \sigma_i^2) \times \mathrm{dNLN}(\alpha_i, \beta_i; \alpha_R, \sigma_\alpha^2, \beta_R, \sigma_\beta^2, \rho)\mathrm{d}\alpha_i \mathrm{d}\beta_i$$

The within-population variance σ_i^2 in eq. 6 are estimated from the *S–R* data from population *i* to reduce parameters (summarized in Table 2). This marginal likelihood function involves two-dimensional integration to α_i and β_i. To simplify this likelihood function, an analytical integration to α_i can be performed from eq. 5 as

$$(7)\quad \int_{\alpha_i} \mathrm{dNorm}(y_{it}; \alpha_i - \beta_i S_{it}, \sigma_i^2) \times \mathrm{dNorm}\left(\alpha_i, \alpha_R + \rho_\tau \frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i - \beta_R), \sigma_\alpha^2(1-\rho_\tau^2)\right)\mathrm{d}\alpha_i = \mathrm{dNorm}\left(y_{it} + \beta_i S_{it}; \alpha_R + \rho_\tau \frac{\sigma_\alpha}{\sigma_\beta}(\ln\beta_i - \beta_R), \sigma_\alpha^2(1-\rho_\tau^2) + \sigma_i^2\right)$$

Therefore, the two-dimensional integration in the marginal likelihood function (6) can be reduced to one-dimensional integration as

$$(8)\quad L(\alpha_R, \sigma_\alpha^2, \beta_R, \sigma_\beta^2, \rho \mid S\text{–}R \text{ data}) = \prod_{i=1}^{K}\prod_{t=1}^{n_i}\int_{\beta_i} \mathrm{dNorm}\left(y_{it} + \beta_i S_{it}; \alpha_R + \rho_\tau \frac{\sigma_\alpha}{\sigma_\beta} \ln\beta_i - \beta_R), \sigma_\alpha^2(1-\rho_\tau^2) + \sigma_i^2\right) \times \mathrm{dLNorm}(\beta_i; \beta_R, \sigma_\beta^2)\mathrm{d}\beta_i$$

The integration to β_i inside eq. 8 is not practical to obtain analytically so a numerical integration is used (Press et al. 1988). Based on eq. 8, the parameter estimates for α_R, σ_α^2, β_R, σ_β^2, and ρ can be obtained either by maximizing eq. 8 or by minimizing the negative log-likelihood function. The implementation of this computation is coded in the S-PLUS language and can be obtained from the senior author.

Results

We first consider the NBC MU (Statistical Area 6; Fig. 2). We fitted the Ricker model (eq. 1) for all 83 populations independently (Table 2). Of the 83 model estimates, 68 of them are significant (*F* test with $p < 0.05$) with an average *p* value of 0.0032 (*p* model in Table 2). When a Kolmogorov–Smirnov test for normality for the residuals was performed, 70 of them had a *p* value greater than 10% with average *p* value of 40% (*p* residuals in Table 2). The average model R^2 is 41% (R^2 in Table 2). Together, all of this information indicates that the Ricker model is appropriate for the coho populations of this area.

Distributional plots of the estimated parameter values indicate that the regional distribution for α_i is normal (Fig. 4*a*), whereas the regional distribution for β_i is lognormal (Fig. 4*b*). The two parameters are highly correlated (Fig. 4*c*). With the newly developed NLN distribution (eq. 3), we can fit the regional meta-model (eq. 2) with the empirical Bayesian approach (eq. 8) (Table 3). For this region, the empirical hierarchical Bayesian model yielded lower variance estimates for both parameters than the simple average and a lower estimate of α_R. With the estimated regional parameters α_R, σ_α^2, β_R, σ_β^2, and ρ, the resultant regional bivariate distribution of the regional parameter estimates for NBC can be generated and plotted (Fig. 6*a*).

The same analysis was followed for the SBC MU. We fit the Ricker model (eq. 1) to the 50 populations independently (Table 2). Of the 50 model estimates, 18 of them are significant (*F* test with $p < 0.05$) with average *p* value as 0.144 (*p* model in Table 2). When a Kolmogorov–Smirnov test for normality for the residuals was performed, 44 of them had a *p* value greater than 10% with an average *p* value of 45% (*p* residuals in Table 2). The average model R^2 is 29% (R^2 in Table 2). Similarly, there are no anomalous patterns to the residuals within the area and we conclude that the Ricker *S–R* model is appropriate for the coho of SBC.

As was the case in NBC, the empirical hierarchical Bayesian model yielded lower variance estimates for both parameters than did the simple average and a lower estimate of α_R than did the simple average of parameter estimates from the Ricker model independently fit to each population. With the estimated regional parameters α_R, σ_α^2, β_R, σ_β^2, and ρ, the resultant regional distribution can be generated and plotted (Fig. 6*a*).

The distribution of a derived management parameter

With the estimated regional distribution defined in eq. 3, the regional distribution for the Ricker α can be easily obtained from the marginal distribution of eq. 3 (see eq. A.3 in Appendix A). This distribution is still normal with mean α_R and variance σ_α^2, i.e., $\alpha \sim N(\alpha_R, \sigma_\alpha^2)$. Therefore, the distribution of MSY exploitation rate can be obtained from the formulation in Hilborn (1985) as $\mu_{\mathrm{MSY}} = \alpha(0.5 - 0.07\alpha)$. This can be easily done by randomly drawing samples from the normal distribution, $\alpha \sim N(\alpha_R, \sigma_\alpha^2)$, and calculating μ_{MSY} for each sample. Sampling distributions for μ_{MSY} and cumulative probability distributions for μ_{MSY} with their respective 25% and 75% quartiles were made after 10 000 random draws (NBC, Figs. 7*a*, 7*c*; SBC, Figs. 7*c*, 7*d*).

Discussion

The use of hierarchical Bayesian frameworks has received considerable attention in the fisheries literature recently summarized by Quinn and Deriso (1999). The use of such estimators has become common in the fisheries literature (e.g., Myers et al. 2001). However, the practitioners of Bayesian approaches have typically assumed the prior distributions of the SR parameters often without much justifica-

Table 2. Summary of the Ricker models fit to the individual populations within northern British Columbia (NBC) and southern British Columbia (SBC).

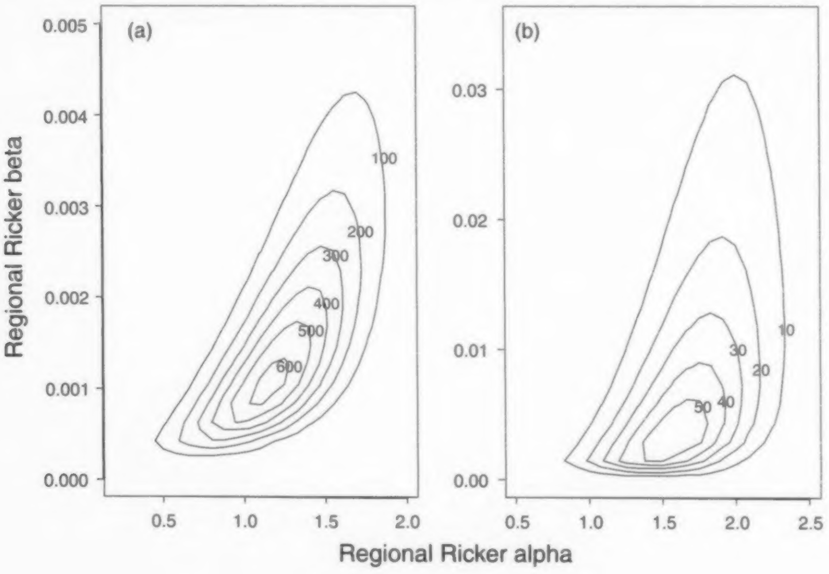
Region	Parameter	α_i	β_i	$\ln(\beta_i)$	σ_i^2	*p* model	*p* residuals	R^2
NBC	Minimum	0.54	2.41×10^{-4}	–8.33	0.06	4.28×10^{-8}	1.79×10^{-3}	8.18×10^{-3}
	Median	1.76	6.82×10^{-3}	–4.99	0.95	4.85×10^{-2}	3.94×10^{-1}	4.11×10^{-1}
	Mean	1.80	9.56×10^{-3}	–5.14	1.00	3.15×10^{-3}	5.00×10^{-1}	4.10×10^{-1}
	Maximum	3.41	3.95×10^{-2}	–3.23	2.64	7.13×10^{-1}	5.00×10^{-1}	9.08×10^{-1}
SBC	Minimum	1.07	1.09×10^{-3}	–2.96	0.12	2.17×10^{-4}	4.55×10^{-3}	2.52×10^{-2}
	Median	2.02	1.80×10^{-2}	–1.74	0.48	6.48×10^{-2}	5.00×10^{-1}	2.54×10^{-1}
	Mean	2.02	4.64×10^{-2}	–1.69	0.54	1.44×10^{-1}	4.48×10^{-1}	2.90×10^{-1}
	Maximum	3.14	2.71×10^{-1}	–0.57	1.64	7.64×10^{-1}	5.00×10^{-1}	7.87×10^{-1}

Note: In this table "*p* model" is the *p* value for the model *F* test and "*p* residuals" is the *p* value for the Kolmogorov–Smirnov test.

Table 3. Estimates of the regional Ricker parameter from the empirical Bayes compared with the simple averages of estimates from model fits to individual populations within northern British Columbia (NBC) and southern British Columbia (SBC).

Region	Method	α_R	σ_α^2	β_R	σ_β^2	ρ
NBC	Simple average	1.790	0.219	–5.135	1.222	0.677
	Empirical Bayes	1.357	0.138	–6.332	0.530	0.643
SBC	Simple average	2.020	0.183	–3.886	1.820	0.802
	Empirical Bayes	1.855	0.165	–4.275	1.548	0.370

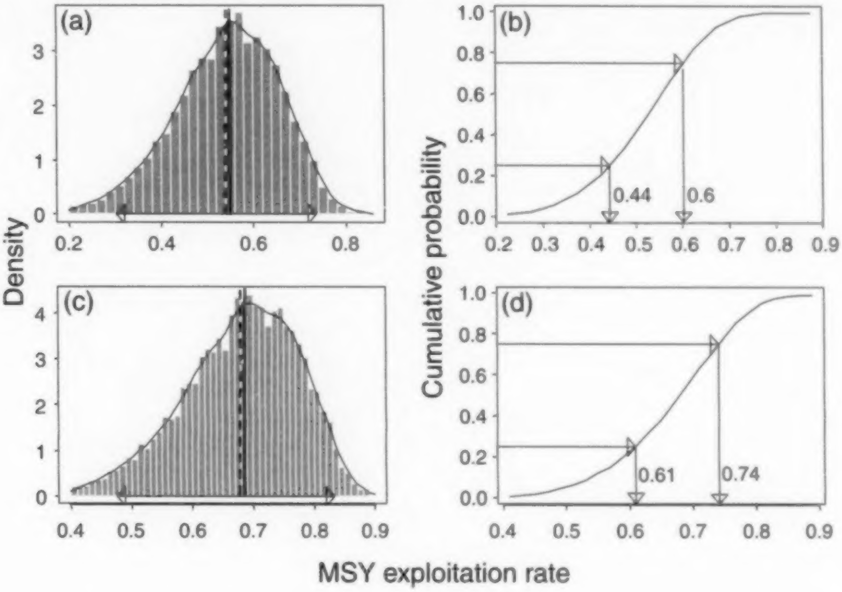
Fig. 6. Contour plots for the joint distribution of regional Ricker α and β for (*a*) northern British Columbia and (*b*) southern British Columbia. The lines with numeric values represent the contour lines of the resultant NLN (normal–lognormal) density.



tion or exploration of alternative prior distributions. To some extent, this practice is understandable because analytical solutions for many plausible prior distributions are not available. For example, it is easy to show that an analytical form for eq. 8 can be easily obtained in the case of a bivariate normal prior distribution. If the prior distribution is really normally distributed, a comprehensive theory for Bayesian analysis can be found from Gelman et al. (1995) and Carlin and Louis (1998). Chen et al. (1999) have developed an empirical Bayesian estimator and also a shrinkage estimator with application to such multivariate situations. Pella et al. (1998) used the same approach in forecasting salmon abundance.

However, Rivot et al. (2001) warn that in *S–R* analysis the Bayesian posterior inferences can be very sensitive to the choice of the prior distributions. Consequently, we began our analysis by estimating the Ricker parameters for each population within the two MUs to provide reasonable regional prior distributions. We found that the regional prior distribution for the two parameters of the Ricker model was bi-

Fig. 7. The probability function and the cumulative probability function of the maximum sustainable yield (MSY) exploitation rate (*a* and *b*, respectively) for the northern British Columbia management region and (*c* and *d*) for southern British Columbia management region. In both (*a*) and (*c*), the curved line on the top of the histogram is the kernel density smoothing estimate. The sample mean and the calculated value for the MSY exploitation rate are marked as vertical broken and solid lines, respectively. The horizontal lines with open arrowheads on both ends indicate the 95% sampling confidence intervals. The horizontal arrows in (*b*) and (*d*) indicate the 25% and 75% quartiles of the estimated distribution of the MSY exploitation rate within each management region.



variate NLN with a high degree of correlation between the two Ricker parameters, a distribution that had not previously been formally described. We would strongly recommend that the selection of the prior distribution should be based on some real data analysis, and if that is not possible, a comprehensive sensitivity analysis should be conducted for different specifications of prior distributions to insure a robust result. In this sense, the approach developed in this paper to derive the regional NLN distribution and the NLN distribution itself can serve as a departure for future Bayesian analysis.

The NLN regional distribution has not been fully developed in the literature. Conceptually, this distribution, which results from applying a log transformation to the second component of a bivariate normal distribution, is one of the distributions in Johnson's translation system (Johnson 1987). To the best of our knowledge, this distribution has not been formally developed; therefore, to pursue our meta-model, we formulated the probability density for the NLN distribution and proved some of its important properties. An empirical Bayesian approach was then used to estimate the regional distributions of the Ricker parameters and derived management parameters of interest.

The estimation procedure in this paper is conducted by reducing the posterior marginal likelihood to a one-dimensional integral. With numerical integration, we estimated the regional Ricker parameters by maximizing the marginal likelihood function. We are currently investigating alternative estimation procedures involving more widely used stochastic methods such as Markov Chain Monte Carlo (Gilks et al. 1996) and nonlinear mixed models (Lindstrom and Bates 1990) and comparing these to the empirical Bayesian approach described in this paper.

We have assumed that the residuals in our *S*–*R* models are independently distributed in both space and time. This assumption is a questionable one. Over the spatial scales of the management regions, we consider it is likely that both freshwater and marine survivals of the component populations are correlated (e.g., Bradford et al. (1997) and Mueter et al. (2002), respectively). We are currently investigating a reformulation of model 2 to incorporating these time and spatial structures within the management region.

The primary objective of this paper was to document a method for describing the distribution of population productivities within a region. The method was illustrated with two examples drawn from coho salmon in British Columbia, and the results are immediately instructive to resource management in several regards. First, the average productivity estimated by averaging estimates from individual populations is higher than the mean of the regional distribution in both MUs. In NBC, the difference in the mean estimated μ_{MSY} is 0.12, which is the exploitation that would result from a substantial ocean troll fishery or many recreational fisheries. Second, characterization of the distributions of the SR and management parameters within the two MUs will prove essential in exploring the spatial distributions of productivity over the MUs contributing to major mixed-stock fisheries. The same distributions are useful in interpreting the declines in abundance that had been observed. Escapement to the NBC streams has been declining since approximately the 1960s. Over the period 1975 to

1997, the average exploitation rate is estimated to have been 65%, with a maximum of 81%. That average exploitation rate exceeds the μ_{MSY} for approximately 87% of the individual populations within the MU and is sufficient to account for the observed decline in overall abundance. Third, the wide range in productivities exhibited by coho salmon populations within each MU means that inferences drawn from "indicator" streams must be made with care. An indicator stream is one where detailed information about life history and production characteristics is collected. Indicator streams are intended to be representative of other populations in the MU.

The wide range of productivities as reflected by MSY exploitation rates and the highly mixed-stock nature of the fisheries that exploit coho underlies the public policy dilemma facing management agencies attempting to define appropriate exploitation levels for coho salmon and for other anadromous species that have similar population structures. A decision to continue with exploitation rates that are appropriate for the productive populations within a MU would quickly reduce many unproductive populations to low levels. However, the effects of such a harvest policy on within-MU diversity could be unacceptable to those whose perspective is confined to a few component populations. This dilemma is compounded when dealing with numerous co-mingling MUs and extensive enhancement. Even within one MU, the problem is potentially more complex if the spatial pattern of productivity within the MU is such that the low productivity systems are clustered.

The trade-off between production (harvest) and diversity is made more difficult when management units of very different mean productivities co-mingle extensively in ocean fisheries. In this situation, it could be difficult to adequately characterize the trade-offs inherent in a particular exploitation rate. Such a situation exists in northern British Columbia where the distribution of coho from Statistical Area 3 (Nass River and Portland Canal), which contains the most productive populations we encountered in B.C. (Holtby et al. 1999), extensively overlaps the distribution of Area 6 (NBC) coho, one of the least productive management units we encountered. The geographies of the two areas are quite different with the streams in Area 6 generally being short and with steep gradients compared with larger and flatter streams in Area 3. Whatever the causes for differing average levels of productivity, the result of that difference has been a geography where coho are slowly becoming rare juxtaposed with an area a scant 100 km away where coho are abundant and becoming more so.

The model developed here enables analyses that can be used to convey to fisheries managers one of the important reasons why extreme contrasts in abundance have developed and, in doing so, encourage more explicit decisions about the setting of exploitation rates in the common fishery that are consistent with desired levels of diversity. The next step in the application of this analysis involves examining in greater detail the spatial and temporal distribution of productivities within and between management unit and the incorporation of environmental information into the model (Chen and Irvine 2001). Wood and Holtby (1998) have demonstrated that in coho salmon the levels of neutral gene flow scale linearly with distance between populations. Loss of diversity or local adaptation might therefore be more severe in situations where many adjacent populations are lost compared with the situation where the same total number of nonadjacent populations is lost. The spatial distribution of productivity within an area of generally low productivity, such as Area 6, and the resolve with which resource managers protect spatial elements of diversity will likely underlie the important fisheries management decisions in the near future.

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Appendix A

Bivariate NLN distribution

Definition of NLN distribution

We sought a bivariate distribution for (X, Y) with two requirements: (*i*) the marginal distribution for X is normally distributed, i.e., $X \sim N(\mu_X, \sigma_X^2)$ and the marginal distribution for Y is lognormally distributed, i.e., $Y \sim \mathrm{LN}(\mu_Y, \sigma_Y^2)$; and (*ii*) X and Y are correlated with the correlation coefficient ρ. If X and Y were independent, then the joint distribution would be the product of the normal density function and lognormal density function. However, it was necessary to incorporate the correlation of X and Y into this joint distribution, because in our meta-model, X (the Ricker productivity parameter α) and Y (the Ricker density-dependent parameter β) are correlated. Conceptually, by applying a log transformation to the second component of a bivariate normal distribution, the desired distribution would be one of the distributions in the Johnson's translation system (Johnson 1987). However, to the best of our knowledge, this distribution has not been fully formulated. Therefore, to pursue our meta-model, we formulated the probability density for this bivariate NLN distribution and proved some of its properties. We have named this distribution the joint bivariate normal–lognormal (NLN) distribution with density function for (X, Y) as

(A.1) $$f_{X,Y}(x, y; \mu_X, \mu_Y, \sigma_X, \sigma_Y, \rho) = \frac{1}{2\pi\sigma_X\sigma_Y\sqrt{1-\rho_\tau^2}y} \exp\left\{-\frac{1}{2(1-\rho_\tau^2)}\left[\left(\frac{x-\mu_X}{\sigma_X}\right)^2 - 2\rho_\tau \frac{x-\mu_X}{\sigma_X}\frac{\ln y-\mu_Y}{\sigma_Y} + \left(\frac{\ln y-\mu_Y}{\sigma_Y}\right)^2\right]\right\}$$

where $-\infty < x < \infty$, $0 < y < \infty$, σ_X and $\sigma_Y > 0$, $-\infty < \mu_X$, and $\mu_Y < \infty$. The additional parameter ρ_τ is related to the correlation coefficient ρ as $\rho_\tau = \dfrac{\rho\sqrt{\exp(\sigma_Y^2)-1}}{\sigma_Y}$ to make eq. A.1 easy to read.

Properties for the NLN distribution

For brevity, we have simply listed the pertinent properties of the NLN distribution without analytical derivations. The detailed mathematical derivation is available on request.

(A.2) $\mu_X = E(X)$, $\sigma_X^2 = \mathrm{Var}(X)$, $\mu_Y = E(\ln Y)$, $\sigma_Y^2 = \mathrm{Var}(\ln Y)$, and $\rho = \dfrac{\mathrm{Cov}(X, Y)}{\sqrt{\mathrm{Var}(X)\mathrm{Var}(Y)}}$

(A.3) Marginal distributions:

$$X \sim N(\mu_X,\ \sigma_X^2) \text{ and } Y \sim \mathrm{LN}(\mu_Y,\ \sigma_Y^2)$$

(A.4) Conditional distributions:

$$X|Y = y \sim N\left(x, \mu_X + \rho_\tau \frac{\sigma_X}{\sigma_Y}(\ln y - \mu_Y), \sigma_X^2(1-\rho_t^2)\right)$$

$$Y|X = x \sim \mathrm{LN}\left(y, \mu_Y + \rho_\tau \frac{\sigma_Y}{\sigma_X}(x - \mu_x), \sigma_Y^2(1-\rho_\tau^2)\right)$$

(A.5) Covariance of X and Y:

$$\mathrm{Cov}(X,Y) = \rho_\tau\sigma_X\sigma_Y\exp\left(\mu_Y + \frac{\sigma_Y^2}{2}\right)$$

Intra- and inter-specific competition for winter concealment habitat in juvenile salmonids

A.J. Harwood, N.B. Metcalfe, S.W. Griffiths, and J.D. Armstrong

Abstract: The availability of streambed refuges has previously been shown to be of critical importance to the survival of overwintering juvenile salmonids. We used semi-natural stream channels to quantify intra- and inter-specific competition for daytime refuges and the willingness of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) to share available shelter. Refuge use was frequently associated with aggressive behaviour when two fish were provided with only one shelter, with intra- and inter-specific competition being similar in intensity. Resident individuals were less likely to leave the refuge than were intruders, and sharing of refuges was uncommon, both when competing for one shelter (experiment I) and when these were provided in excess (experiment II). Fish showed greater preferences for foraging habitat during the night than for the location of daytime shelters and were therefore willing to shift habitats to find adequate shelter. Overall, these results suggest that winter competition for refuges, both within and between species of salmonid, is likely to be intense if refuge availability is limited in the wild.

Résumé : Il a été démontré que la disponibilité de refuges dans le lit du cours d'eau est un facteur critique pour la survie des jeunes saumons en hiver. Des ruisseaux semi-naturels nous ont servi à quantifier la compétition intra- et inter-spécifique pour des refuges de jour et d'évaluer dans quelle mesure des saumons de l'Atlantique (*Salmo salar*) et des truites brunes (*Salmo trutta*) sont disposés à partager les refuges disponibles. Lorsque deux poissons sont en présence d'un seul refuge, son utilisation génère souvent des comportements agressifs d'intensité semblable, qu'il s'agisse de compétition intra- ou inter-spécifique. Les résidents sont moins susceptibles d'abandonner le refuge que les intrus et le partage d'un refuge reste rare, qu'il y ait compétition pour un même refuge (expérience I) ou qu'il y ait un surplus de refuges (expérience II). Les poissons montrent plus d'intérêt pour les substrats de nuit de quête de nourriture que pour les refuges de jour et ils sont donc disposés à changer d'habitat pour trouver un refuge adéquat. Généralement, nos résultats laissent croire qu'en nature, la compétition pour les refuges en hiver est probablement intense lorsque la disponibilité des refuges est limitée, tant au sein d'une espèce qu'entre les espèces de salmonidés.

[Traduit par la Rédaction]

Introduction

Sheltering behaviour is widespread among both invertebrates and vertebrates and can be used to investigate the trade-off between the cost of lost feeding opportunities and the benefit of predator avoidance during an individual's decision-making processes (Dill and Fraser 1997; Krause et al. 1998). For example, Dill and Fraser (1997) were able to measure the cost of sheltering in terms of lost growth in the polychaete worm, *Serpula vermicularis*, which filter-feeds at the mouth of its calcareous tube but retreats when threatened. Shelter use can influence an individual's chance of survival, particularly through a decreased risk of inter- and intra-specific predation. Predator avoidance has been cited as the primary function of sheltering behaviour in salmonid fishes (Gregory and Griffith 1996*a*; Valdimarsson and Metcalfe 1998). However, in nature, the ability to find a suitable refuge can also have other benefits. For example, Smith and Griffith (1994) found that rainbow trout (*Oncorhynchus mykiss*) survival in winter was higher in enclosures with cobble substrate than in those without, even though both enclosures excluded predators. The presence of rocks in the enclosures allowed trout to shelter in interstitial spaces that provided them not only with protection against physical damage from dislodged ice moving in the water column, but also with a reduction in daytime energy expenditure. The ability to find adequate shelter can therefore influence survival under natural conditions, independent of predation.

Winter can be a bottleneck during which density-dependent processes occur in some populations of salmonid fish (Bjornn 1971; Mason 1976), with availability of suitable habitat being cited as one of the main limiting factors (Rimmer et al. 1985;

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Nickelson et al. 1992; Cunjak 1996). There are two types of preferred winter habitat important to the survival of fishes. First, slow-flowing water has been shown to be important for overwintering salmonids (e.g., Heggenes et al. 1993; Whalen and Parrish 1999; Harwood et al. 2001). Secondly, cobble–boulder substrates that provide shelter have been found to be of critical importance for both stream-dwelling salmonids and cyprinids (see Cunjak 1996). Habitat-enhancement programmes have highlighted the importance of both habitats. Solazzi et al. (2000) have shown that the addition of large woody debris to newly created dammed pools provides salmonids with refuge from high-velocity conditions and improves overwintering survival of coho salmon (*Oncorhynchus kisutch*), cutthroat trout (*Oncorhynchus clarki*), and rainbow trout, as well as increasing coho salmon smolt production. Similarly, the addition of patches of cobble to a stream in Idaho increased the number of overwintering chinook salmon (*Oncorhynchus tshawytscha*) eightfold over numbers from the previous winter, despite numbers being comparable at the onset of the two winters (Hillman et al. 1987).

The importance of shelters in salmonid ecology is not restricted to the winter months, as some species of salmonid also shelter during the day in summer (Rimmer et al. 1984; Gries et al. 1997; Gries and Juanes 1998). Despite the significance of shelters, few studies have examined whether fish actively compete for, or defend, refuges. This is largely because fish are thought to become less aggressive during winter (McMahon and Hartman 1989; Fraser et al. 1993) and studies in summer have concentrated on territorial disputes over food rather than shelter resources (e.g., Fausch 1984). McMahon and Hartman (1989) and Glova (1986) both reported territorial behaviour among cover in juvenile coho salmon and cutthroat trout, respectively; however, only Gregory and Griffith (1996*b*) have quantified the aggression associated with shelter use.

Here we report more detailed investigations into competition for refuges than have previously been conducted. In our first experiment, where shelter availability was limited, we provide the first quantitative assessment of shelter competition among conspecific and heterospecific pairs of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). We test for asymmetry in the abilities of Atlantic salmon and brown trout to compete for shelter during winter. Aggressive behaviour and shelter sharing were observed at dawn, when movement into shelters is at its peak (Gregory and Griffith 1996*b*). In experiment II, when shelters were provided in excess, we examined the frequency of shelter sharing among allopatric groups of salmon and sympatric groups of Atlantic salmon and brown trout. The influence of surrounding habitat type on refuge use was also investigated.

Materials and methods

Experiment I

This experiment was carried out in a glass stream-channel system at the University of Glasgow, U.K. The channel consisted of a stack of three glass tanks measuring 180 cm × 25 cm × 20 cm and one glass tank measuring 120 cm × 25 cm × 20 cm, which were interconnected by plastic pipes. These tanks then drained into two 60-L sumps, the water from which was recirculated by pumping it into the top stream tank. The stream-channel system was positioned within a temperature-controlled laboratory. In order to separately control the lighting regime for each glass tank, the system was surrounded by black polythene sheeting to block out any light from the laboratory. An observation hide was also created so that fish could be observed without casting any external light on the tanks. The glass tanks had a 4-cm layer of gravel substrate, a water depth of 11 cm, and a flow rate of approximately 0.03 $m \cdot s^{-1}$. Partitions placed within the tanks created compartments; the partitions were opaque but had panels of fine mesh to allow a directional current through the tanks. The compartments in which the experiments were conducted, hereafter referred to as experimental arenas, measured 40 cm × 25 cm. Six experimental arenas were used in total, two in each of the longer glass tanks. Although juvenile salmonids are unlikely to occupy such a simplified habitat in nature, refuge availability is a limiting factor for overwintering survival in the wild (e.g., Hillman et al. 1987; Griffith and Smith 1993; Cunjak 1996).

A feeder and a shelter were placed in opposite corners of each arena so that fish could not feed while in the shelter. The feeders consisted of 25-mL transparent plastic tubes, each with a small hole drilled near the top to allow water to circulate and a small hole (1.5 mm in diameter) near the bottom to allow a mixture of live *Daphnia* and chironomid larvae to escape at random after having been placed in the feeder. A plastic mount held each tube so that the exit hole for the larvae was 5.5 cm above the substrate. The shelters were made from 1-L opaque plastic bottles cut lengthwise into quarters and embedded in the gravel, creating cavities in the gravel measuring 15 cm × 4 cm × 4 cm. Fish could enter the shelters through a 4 cm × 4 cm vertical hole that was cut into the top corner of each shelter and was positioned flush with the surrounding gravel. These shelters were placed at the downstream end of each section with the open side against the glass wall of the tank, allowing any fish within them to be identified (see Valdimarsson et al. 1997).

The fish used were wild-caught age-0+ Atlantic salmon (fork length = 65.5 ± 1.07 mm (mean ± standard error (SE)); weight = 2.64 ± 0.13 g) and brown trout (fork length = 76.7 ± 1.62 mm; weight = 4.28 ± 0.28 g) from the River Endrick, which flows into Loch Lomond, west Scotland. Fish were captured during a single day less than 1 week before experiments began and were kept in tangential-flow holding tanks, except when being tested. These fish were used in one of three treatments. Allopatric trials (either two salmon or two trout) were used to examine intra-specific competition for shelters in each species, while sympatric trials (one salmon and one trout) were used to study interspecific competition for shelters. Fish in all three treatments were approximately size-matched (size differential < 20%) to ensure that they had similar habitat preferences and so would compete for the refuge. After anaesthetization using benzocaine, fish were individually marked on their caudal fins using small injections of Alcian Blue dye. They were then placed in experimental arenas on the evening before observations began, and light intensity was reduced to night-time levels (0.01 lx) 30 min later. To ensure that aggressive interactions during initial shelter acquisition were noted, observations began the following day. Light intensity was recorded using a photometer (SKL 300, range 0.01–2000 lx

(Skye Instruments Ltd., Powys, U.K.)), placed alongside the experimental arena.

Light intensity was controlled by the use of three dimmer switches, each of which controlled two bulbs that were situated above the two experimental arenas located on each shelf. To simulate natural dawn conditions, light levels were gradually increased by 33 lx every 5 min for 45 min, reaching a maximum of approximately 300 lx. The position of each fish (either in or out of shelter) was recorded immediately before the dawn light manipulation and then every 5 min over the 45-min dawn observation period. During this same period, continuous observations of feeding attempts, aggressive behaviour, and movement in and out of the shelter were made. For each aggressive interaction, the location and identity of aggressor and recipient was noted (see Kalleberg (1958) for a description of aggressive behaviour among juvenile salmonids). Any movement by the recipient between habitats was also noted (fish were defined as being forced to move habitats if they moved in or out of shelter within 5 s of having been attacked).

Because only two arenas could be monitored simultaneously, it was necessary to stagger the timing of the simulated dawn so that data could be obtained from all six arenas each day. Fish experienced dawn a maximum of 90 min after natural dawn, and the sequence in which treatments were observed was randomized to minimize any effects that shifting photoperiod may have had on the fish. After the dawn period, light levels were kept constant at approximately 300 lx for 8 h in each tank, after which light levels were reduced directly to 0.01 lx until "dawn" the next morning. The fish were fed 1% of their body weight per day, with the feeders being replenished with *Daphnia* and chironomids immediately before light levels were reduced in the evening and immediately after all observations had been carried out in the morning. Equal amounts of food were given in the morning and in the evening.

Observations were made on four successive mornings, after which time the fish were removed and replaced by new groups. All three treatments were replicated eight times, with all fish being used only once. Water temperature was continuously measured using a digital thermometer placed in the stream tank and varied between 6.9°C and 10.3°C during the course of the experiment, which ran between 7 February and 8 March 2001. We were unable to conduct experiments I and II under the same temperature regime; however, both experiments were conducted within the range of naturally occurring winter temperatures in Scotland and below the critical value of 10°C at which juvenile salmonids switch from diurnal to nocturnal behaviour (Fraser et al. 1993). We therefore have no reason to believe that differences in temperature regimes influenced the direction of our results.

Experiment II

A full description of the materials and methods for this experiment is given in Harwood et al. (2001), and only details relevant to shelter use are presented here. A comparison of the experimental procedure of experiments I and II can be seen in Table 1. Experiment II consisted of monitoring habitat and shelter use by salmon and trout in a semi-natural outdoor stream channel at the University Field Station, Rowardennan, Scotland. In a series of replicated trials, groups of four salmon (allopatric treatment) and two salmon with two trout (sympatric treatment) were observed in identical experimental arenas, each of which had a gradual increase in water depth from 10 cm at the upstream end to 45 cm at the downstream end. Accordingly, the surface velocity of the water varied continuously from 0.16 $m \cdot s^{-1}$ at the upstream end to 0.03 ms^{-1} at the downstream end. Each experimental arena was 225 cm × 60 cm, and the outer side of the channel was marked every 15 cm to define 15 zones per arena (with zone 1 being at the upstream end) and allow referencing of the recorded positions of each fish. The arenas were landscaped with fine gravel (5–25 mm diameter) to prevent fish from hiding in streambed cavities. Six shelters were placed at regular intervals in each of the experimental arenas with the open side against the glass wall to allow any fish within them to be identified by their Alcian Blue marks (see below). The shelters were made from 1-L opaque bottles that were cut in half lengthwise, creating cavities in the gravel measuring 17 cm × 8.5 cm × 4.25 cm. Hence, in this experiment, streambed shelters were provided in excess.

Table 1. Summary of the experimental design for the two experiments.

	Experiment I	Experiment II
Number of individuals/replicate	2	4
Number of shelters/replicate	1	6
Habitat	Simple	Varied
Settling time	None	72 h
Number of days observed	4	4
Treatments		
Salmon in allopatry	Y (8)	Y (8)
Trout in allopatry	Y (8)	N
Salmon and trout in sympatry	Y (8)	Y (8)

Note: Numbers in parentheses represent the number of replicates.

Water was pumped constantly from Loch Lomond into the stream channel and ranged in temperature from 4.3 to 6.1°C. Light conditions and photoperiod for the outdoor stream channel were the same as the ambient conditions of Loch Lomond. Fish were provided with 0.5% of their total body weight in food per day. The food was delivered via a belt feeder that dropped pelleted food at a trickle rate at the upstream end of each arena. The fish used were wild-caught age-1+ Atlantic salmon (fork length = 109.4 ± 1.83 mm (mean ± (SE)); weight = 13.42 ± 0.65 g) and brown trout (fork length = 100.7 ± 2.77 mm; weight = 9.87 ± 0.90 g) from the River Blane, a tributary of the River Endrick. Fish were captured during a single day 1 week before the start of the experiments and were kept in tangential-flow holding tanks, except when being tested. The fish were approximately size-matched (size differential < 20%) and individually marked as in Experiment I. Fish were then allowed to reside in the experimental arenas for a period of 72 h before observations began.

Observations on each group of acclimated fish were made on four consecutive dates. Each observation period lasted 7 h and covered the day–night transition at either dusk or dawn. During each observation period, the position of each fish was recorded every 30 min by scanning briefly with a flashlight, a method chosen to minimize the disturbance of the fish (see Heggenes et al. 1993). Data collected during each scan observation included the zone number in which each fish was located (1–15) or the shelter in which the fish was hiding (1–6, with shelter 1 being at the upstream end). Light intensity and water temperature were also noted. Light intensity was measured using a SKL 300 photometer (range 0.01–2000 lx, Skye Instruments) and recorded as the mean of two measurements made just above the water surface. Water temperature was measured using a digital thermometer placed permanently in the stream. After the data for each group were collected, the fish were removed and different fish were placed in the test arena. Both treatments were replicated eight times; all fish were used only once. The experiments were carried out between 16 January and 4 March 1999.

Data analysis and statistical treatment

Experiment I

To determine whether fish increased their use of shelter as the simulated dawn period progressed, we calculated the mean percentage of observations in which each individual fish was in shelter over the 4 days at the start point (dimmest) and end point (brightest) of each observation period. The mean for each replicate was then calculated and used in a paired-sample *t* test. To examine the occurrence of shelter sharing in the different treatments, we calculated the percentage of all observations in which both fish were found occupying the shelter. We used one-way analysis of variance (ANOVA) using replicate mean values as data points to determine whether there were any species or treatment effects on the percentage of time that fish spent sharing shelters. Analysis of covariance (ANCOVA) was used to determine whether absolute size (averaged for the two fish within a replicate), or the size difference between the two fish in a replicate, had any effect on the amount of time that fish spent sharing shelters.

The analyses described above were used to investigate overall trends in shelter use; therefore, when calculating the replicate means in the sympatric treatment, the two species were not treated separately. However, when calculating replicate means for all subsequent analyses, salmon and trout in sympatry were treated separately in order that the behaviour of each species under allopatric and sympatric conditions could be compared. Fish were therefore divided into the following categories: salmon in allopatry, trout in allopatry, salmon in sympatry (with trout), and trout in sympatry (with salmon). Two-way ANOVA was used to determine which species (trout or salmon) spent most time in shelter under allopatric conditions and to ascertain whether the larger fish of a pair was able to monopolize shelter. The percentage of observations in shelter over the whole trial was the dependent variable and size (larger or smaller of a pair) and species (salmon or trout) were the fixed factors. Paired-sample *t* tests were used to test for similar relationships in the sympatric trials.

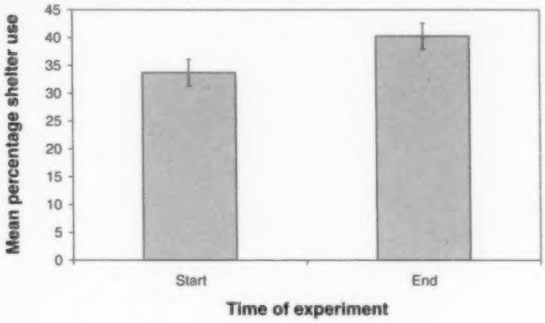
Data on aggression were analyzed by calculating the rate of aggression per minute for each individual and then calculating replicate means, while maintaining the integrity of species data in the sympatric trials. The rate of aggression both in and out of the shelter was also calculated, controlling for the time that fish spent in different habitats by using only observation periods in which both fish started in the same habitat (either in or out of shelter). Separate two-way ANOVAs were used to determine whether there was any difference between allopatry and sympatry in the percentage of aggressive interactions that forced a habitat switch in salmon and trout. The percentage of aggressive interactions that forced a habitat switch was the dependent factor with category of fish (allopatry or sympatry) and the direction of enforced change (into or out of shelter) as the fixed factors. To determine whether there was a prior residence effect on shelter use, the number of occasions when a fish entered an already occupied shelter was counted along with the percentage of such times when the intruder left before the resident, and vice versa. The relationship between the percentage of times that the intruder left before the resident and the relative length of the intruder (its body length as a percentage of that of the resident) was investigated using linear regression. Finally, as a result of the temperature fluctuations that occurred over the course of the experiment, regressions were carried out to determine whether temperature had an effect on the rate of aggression or the percentage of time that fish spent sharing shelters.

Experiment II

A comparison of the overall percentage of time that fish spent sharing in allopatry and sympatry was undertaken in a manner identical with that of Experiment I, by calculating overall replicate means. The percentage of time spent sharing with the same or different species during the sympatric trials was calculated by taking a mean for the two salmon in a replicate and a mean for the two trout, thus preserving the integrity of the species data. When determining whether fish preferred to share with the same or different species in sympatry, it was necessary to control for the number of fish of each species type. Because there were four fish in each replicate trial, each fish had a one in three chance of sharing with a conspecific shelter-mate and a two in three chance of sheltering with a heterospecific shelter-mate. Therefore, the percentage of time spent sharing with conspecifics was multiplied by one-third, whereas the percentage of time spent sharing with the heterospecifics was multiplied by two-thirds. These values were then compared using a Mann–Whitney *U* test. A Wilcoxon's signed-rank test was used to determine whether salmon shared with other salmon more frequently than trout shared with other trout.

For the purpose of comparing the relative use of different habitats for foraging and for shelter, zones were divided into those in deep water (zones 11–15), shallow water (zones 1–5), and water of intermediate depth (zones 6–10). The six shelters were categorized in a similar manner with shelters in deep (5 and 6), intermediate (3 and 4), and shallow (1 and 2) water. Analysis of covariance was then used to examine the relationship between the percentage of time in deep shelters (dependent factor) and the percentage of foraging (i.e., nonsheltering) time in deep zones (covariate), with category of fish (salmon in allopatry; salmon in sympatry; trout in sympatry) as the fixed factor. To determine the relative importance of using shelters located in deep water and the use of deep water for foraging, we used a Wilcoxon's signed-rank test to compare the percentage of observations of sheltering in which an individual was in deep shelters with the percentage of foraging observations in which an individual was recorded in deep zones.

Fig. 1. Mean percentage of observations (± standard error) spent in shelters for individuals from all treatments at the start and end of daily dawn observation periods in Experiment I. Percentages are arcsine-transformed, thus a value of 45 corresponds to equal amounts of time spent in and out of shelter.



For both experiments, all percentage data were normalized by arcsine transformation before use in parametric tests. On the occasions when transformation did not normalize the data, nonparametric tests were used. All quoted probabilities are for two-tailed tests of significance.

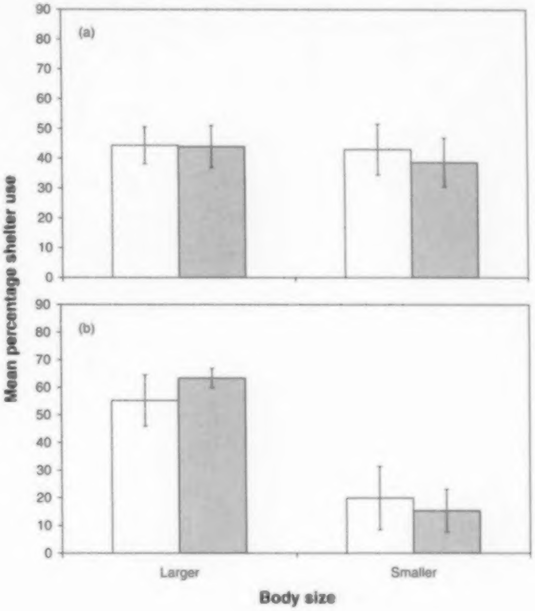
Results

Experiment I

There was a significant increase in the use of shelters from the start to the end of the simulated dawn period when considering all replicates of the three treatments (paired-sample *t* test: $t_{23} = 2.56$, $P = 0.018$; Fig. 1), indicating that the increase in light intensity at dawn prompted fish to seek shelter. There were no significant differences between species or treatments in the percentage of time over the dawn observation period that fish spent sharing shelters (one-way ANOVA: $F_{2,23} = 1.06$, $P = 0.364$; salmon in allopatry, 21.7 ± 5.27% (mean ± SE); trout in allopatry, 18.9 ± 6.39%; sympatry, 10.4 ± 5.30%). There was a significant trend for larger salmon in allopatry and trout in allopatry to share more often than smaller salmon and trout. However, when the two species occurred together in sympatry, larger fish shared less often than smaller fish (ANCOVA, comparison of regression slopes: $F_{2,18} = 4.86$, $P = 0.020$). There was, however, a lot of noise in the regression equations with the relationship for trout in allopatry in particular being driven by one outlier. There was no significant relationship between the percentage of time that the shelter was shared and the weight difference between the two fish in a replicate (ANCOVA: comparison of regression slopes, $F_{2,18} = 2.97$, $P = 0.077$; effect of weight difference, $F_{1,20} = 0.14$, $P = 0.715$; comparison of regression elevations (effect of treatment), $F_{2,20} = 1.01$, $P = 0.384$).

There was no significant difference between categories of fish in the rate at which fish were observed to enter the shelter (one-way ANOVA: $F_{3,31} = 0.37$, $P = 0.773$). There was no effect of an individual's body size (two-way ANOVA: effect of size, $F_{1,29} = 0.20$, $P = 0.657$) or species (effect of species, $F_{1,29} = 0.11$, $P = 0.747$) on the percentage of observations that fish in allopatry were found in shelter (Fig. 2*a*). However, in sympatry, there was a significant effect of relative body size (paired-sample *t* test: $t_7 = 4.47$, $P = 0.003$), with the larger fish in a pair spending more time in the shelter over the dawn period than the smaller fish, although there was no difference between the two species (paired-sample *t* test: $t_7 = 0.68$, $P = 0.518$; Fig. 2*b*).

Fig. 2. Shelter use in Experiment I in relation to relative body size. Mean percentage of time (± standard error) spent in shelters for the larger and smaller fish in a pair for (*a*) allopatric salmon (*Salmo salar*, open bars) and trout (*Salmo trutta*, shaded bars) and (*b*) sympatric salmon (open bars) and trout (shaded bars). Percentages are arcsine-transformed.



There was no significant difference in the rate of aggression by salmon and trout, either in allopatry or sympatry (one-way ANOVA: $F_{3,31} = 1.84$, $P = 0.163$). There was also no significant difference in the amount of aggression that was initiated in, vs. out of, shelter (nonparametric two-way ANOVA, Scheirer–Ray–Hare test (Dytham 1999): effect of being in or out of shelter, $\chi^2 = 0.93$, df = 1, $P = 0.761$; effect of category of fish, $\chi^2 = 1.58$, df = 3, $P = 0.663$; interaction between category and position, $\chi^2 = 0.41$, df = 1, $P = 0.998$). For both salmon in allopatry and salmon in sympatry, a higher percentage of aggressive interactions forced fish out of shelters than forced fish into shelter (two-way ANOVA: effect of category of fish, $F_{1,17} = 0.12$, $P = 0.731$; effect of context of aggression, $F_{1,17} = 12.53$, $P = 0.003$; Fig. 3*a*). A similar result was found for trout in both allopatry and sympatry (two-way ANOVA: effect of category of fish, $F_{1,20} = 0.18$, $P = 0.679$; effect of type of aggression, $F_{1,20} = 7.43$, $P = 0.013$; Fig. 3*b*).

The first fish to enter the shelter during an observation period was recorded on 80 occasions, and in 55 of these cases, this first fish was the larger of the pair, a significantly higher proportion than would be expected by chance ($\chi^2 = 10.51$, df = 1, $P < 0.01$). There was no significant difference between treatments in the percentage of times that the larger

Fig. 3. Consequences of aggression in Experiment I. Mean percentage (± standard error) of the aggressive interactions occurring when both fish were outside of the shelter that led to one fish being forced into shelter and percentage of aggressive interactions that occurred when both fish were inside the shelter that forced one fish out for (*a*) allopatric (shaded bars) and sympatric salmon (*Salmo salar,* solid bars) and (*b*) allopatric (shaded bars) and sympatric trout (*Salmo trutta,* solid bars). Percentages are arcsine-transformed, thus a value of 90% represents a situation where aggression always forced the other fish to shift habitats.

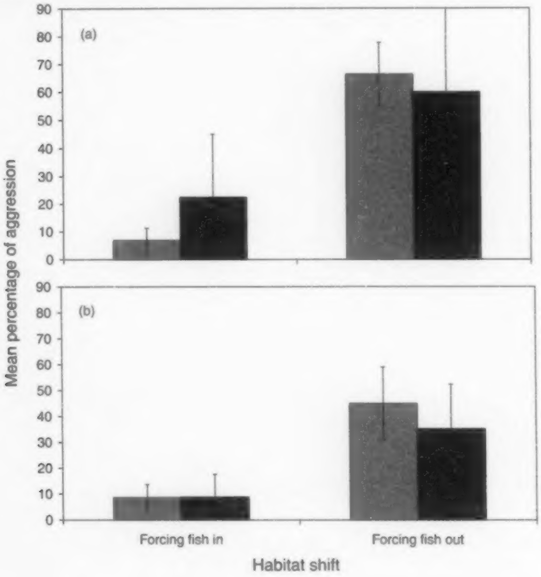


Fig. 4. Percentage of times in Experiment I that the intruding fish, on entering an already occupied shelter, then left before the prior resident. Percentages are plotted with respect to the relative length (expressed as percentage size difference from the resident) for fish in allopatry (solid diamonds, solid regression line, see text for analysis) and fish in sympatry (shaded squares, broken line). Percentages are arcsine-transformed, thus intruders with a value of 90% always left the shelter before the resident.

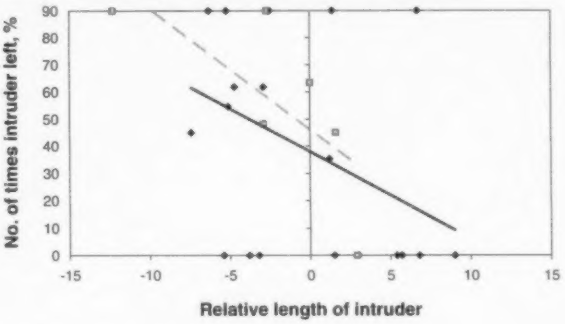
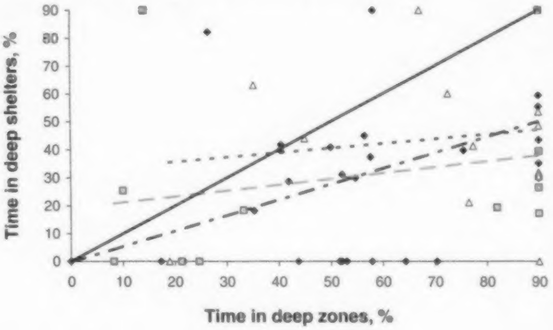


Fig. 5. Percentage of observations of sheltering in Experiment II in which the fish was in deep shelters against percentage of observations of the same fish out of shelter in which it was in deep zones. Data are plotted separately for salmon (*Salmo salar*) in allopatry (solid diamonds; dashed–dotted line), salmon in sympatry (shaded squares; dashed line), and trout (*Salmo trutta*) in sympatry (open triangles; dotted line). Solid black line represents equal percentage of observations in deep shelters and deep zones; percentages are arcsine-transformed.



fish entered first (one-way ANOVA: $F_{2,21} = 2.11$, $P = 0.146$), although there was a trend for the larger fish in sympatry to enter first on more occasions (75.7 ± 7.5%, mean ± SE) than the larger fish in allopatry (salmon in allopatry, 43.8 ± 14.8%; trout in allopatry, 53.7 ± 10.2%). On entering an already occupied shelter, the intruder left first significantly more times than did the resident (percentage of occasions that intruder left first: salmon in allopatry, 76.6%; trout in allopatry, 75.0%; sympatry, 77.8%; $\chi^2 = 21.78$, df = 1, $P < 0.001$). Size was also a factor in predicting which fish left first, with larger intruders less likely to leave before the resident than smaller intruders (regression: $r^2 = 0.23$, $F_{1,24} = 7.34$, $P = 0.012$; Fig. 4).

Temperature had no significant effect on either the rate of aggression (regression: $r^2 = 0.019$, $F_{1,94} = 1.79$, $P = 0.185$) or the percentage of fish sharing shelters (regression: $r^2 = 0.002$, $F_{1,94} = 0.16$, $P = 0.694$), possibly because of the narrow temperature range during the experimental period.

Experiment II

There was no significant difference between the allopatric and sympatric treatments in the percentage of time that fish spent sharing (Mann–Whitney *U* test: $U = 21.0$, $z = -1.16$, $P = 0.244$). In sympatry, neither salmon nor trout showed a preference for sharing with conspecific over heterospecific shelter-mates (Mann–Whitney *U* test: $U = 29.0$, $z = -0.38$, $P = 0.702$). There was also no significant difference in the sympatric treatment between the amount of time salmon spent sharing with salmon and the amount of time trout spent sharing with trout (Wilcoxon's signed-rank test: $z = -0.447$, $P = 0.655$).

There was a significant positive relationship between the percentage of observations where an individual fish was recorded in deep zones when out of refuges (presumed foraging) and the percentage of observations where fish were occupying shelters in deep water, for all categories of fish (ANCOVA: comparison of regression slopes, $F_{2,49} = 0.88$, $P = 0.423$; effect of zone use, $F_{1,51} = 7.46$, $P = 0.009$; comparison of regression elevations, $F_{2,51} = 0.30$, $P = 0.743$; Fig. 5). However, the preference for deep zones when out of shelters was stronger than the preference for deep shelters. Thus the percentage of foraging observations in which an individual was recorded in deep zones was generally greater than the percentage of observations of sheltering in which it was recorded in deep-water shelters (Wilcoxon's signed-rank test: $z = -4.461$, $P < 0.001$).

Discussion

Our experiments show that Atlantic salmon and brown trout compete for daytime shelters during winter and that intra- and inter-specific competition can be equal in intensity. Experiment I shows that there was a prior residence effect involved in the resolution of contests over shelters, and experiment II suggests that fish were willing to shift habitats to find adequate shelter. These findings and the implications of this competition are discussed below with reference to other relevant work.

Fish of both species increased their use of shelters during dawn as light intensity increased in experiment I, as predicted based on previous work in which salmonids have been found to be active at night but then seek shelter during daylight in winter (e.g., Fraser et al. 1993; Heggenes et al. 1993). The frequency with which fish shared shelters was low in both of our experiments, supporting previous studies on salmonid sheltering behaviour in which the use of refuges was almost exclusively by single fish both in winter (Glova 1986; Cunjak 1988; Gregory and Griffith 1996*a*) and in summer (Rimmer et al. 1984; Gries and Juanes 1998). Armstrong and Griffiths (2001), using wild fish in a semi-natural stream channel, found that shelter use was density dependent, with the mean number of fish per refuge being ≤1.5 even at densities that would result in five fish per shelter if all fish were sheltering simultaneously. Most previous work therefore shows that salmonids do not share shelters with conspecifics, suggesting that intra-specific competition for shelters would arise if refuges were limited in number.

There was no difference in either of our experiments in the frequency with which fish shared shelter with members of the same and different species, suggesting that shelters were of equal importance to both species and that intra- and inter-specific competition for shelters was equivalent in intensity. This is supported by the fact that salmon and trout initiated similar levels of aggression in experiment I, both in allopatry and sympatry. This result is surprising as trout are normally considered to be more aggressive than salmon of a similar size (Kalleberg 1958; Kennedy and Strange 1986) and may reflect the importance of shelter to both species. The rate of aggression witnessed in this study (overall mean of 0.030 interactions per fish per minute in experiment I) was similar to the rate observed between rainbow trout competing for shelters in a laboratory stream (Gregory and Griffith 1996*b*). Although these rates of aggression are lower than those reported for salmonid populations in summer (e.g., Hartman 1965) and the onset of winter is associated with a reduction in aggressive behaviour associated with feeding territories (Fraser et al. 1993; Heggenes et al. 1993), individuals still compete for shelters. We have recently shown that intra- and inter-specific competition for deep, slow-flowing water also occurs in winter (Harwood et al. 2001), indicating that competition for limited resources occurs throughout the year, not simply during the summer when individuals compete for food (e.g., Fausch 1984).

Aggression inside shelters during experiment I was commonly followed by one fish then moving outside, whereas aggression out of shelters did not usually lead to either fish entering a shelter. This suggests that aggressive attacks within a shelter served to drive a competitor out. Gregory and Griffith (1996*b*) also provide indirect evidence that the majority of aggression that they observed was related to competition for shelters, because significantly more aggressive acts occurred during the initial concealment period (first 90 min of daylight) than during the rest of the day.

Competition for shelters among underyearling rainbow trout was related to the size of individuals, with one of the larger fish within a group defending a shelter and initiating almost 80% of the aggression (Gregory and Griffith 1996*b*). In experiment I, individual Atlantic salmon and brown trout did not monopolize shelters or perform aggressive acts in such a manner under allopatric conditions. However, when in sympatry, the largest individual tended to gain a disproportionate share of the shelter, irrespective of its species identity. These contrasting results may be explained by the prior residence effect which dictates that a holder of a territory or shelter has an advantage over intruders and is more likely to win contests for that resource (e.g., Blank and Figler 1996; Tobias 1997). Evidence for a prior residence effect on shelter use was witnessed in all three treatments, with the intruder to an occupied shelter leaving first significantly more often than the resident did. This prior residence effect was related to body size, with relatively larger intruders less likely to leave before the resident than were smaller intruders. Although there was no significant difference in the mean percentage of times that the larger fish of a pair entered first in the different treatments, there was a trend for the larger fish to enter first more often in sympatry than in allopatry. The tendency for the larger fish in sympatry to enter the shelter first (and so obtain an advantage over intruders) may explain why the largest fish in sympatry was able to monopolize shelter use, regardless of species identity. In contrast, in both allopatric treatments, there was a lot of variation in the percentage of times that the large fish entered first, and this may explain why large fish were unable to monopolize shelter use in this situation. Blank and Figler (1996) have previously shown prior residence effects to be important in resolving inter-specific competition for shelters between two sympatric species of crayfish (*Procambarus clarkii* Girard and *Procambarus zonangulus* Hobbs and Hobbs). Further work is needed to determine the importance of prior residency in determining competitive advantage for shelter use in Atlantic salmon and brown trout, as the residency effects witnessed in experiment I were established even though fish were introduced to the arena simultaneously.

Evidence from our second experiment, where fish had a choice of habitats, shows that there was a positive relationship between the relative proportion of foraging time that individual fish spent in their favoured deep, slow-flowing water (see Harwood et al. 2001) and the proportion of sheltering time spent in shelters within that habitat. However, the fact that the relative proportion of foraging time spent in deep water was greater than the equivalent proportion of sheltering time spent in deep-water shelters suggests that fish are willing to move to find shelters or foraging habitat. A certain level of activity and continued movement by salmonids in winter may be adaptive to avoid periodic flooding, freezing, and thaws (Cunjak 1996; Whalen et al. 1999). For instance, Whalen et al. (1999) showed that a period of ice formation led to an extensive alteration in the habitat used by Atlantic salmon parr.

Implications

Intra- and inter-specific competition for shelters (Gregory and Griffith 1996*b*; this study) can lead to density-dependent use of refuge habitat (Armstrong and Griffiths 2001) that could have important implications in terms of the carrying capacity for wild populations of salmonids in streams. In some instances, the availability of instream refuges has been found to be high relative to the number of parr, with disturbed individuals being able to find new shelters easily (Cunjak 1988). Such abundance of shelters relative to the requirements of the population of fish may result in no density-dependent mortality over the winter period (Egglishaw and Shackley 1977; Cunjak et al. 1998). However, in other studies, suitable cobble–boulder substrate has been shown to be both limiting (e.g., in areas where the stream flows over the bedrock) and crucial to salmonid survival (Hillman et al. 1987; Griffith and Smith 1993), with individuals emigrating from areas where adequate cover was not available (Bjornn 1971). Under these circumstances, the number of individuals surviving winter may indeed depend on the availability of refuges.

For stream managers wishing to increase productivity of fish populations by habitat manipulation, it is important to understand competition for habitat among species (e.g., Harwood et al. 2001). Increased production of one species during summer months resulting from the addition of shelter may be lost if that species is excluded by another one during winter. This study suggests that when in sympatry, neither brown trout nor salmon of similar size consistently wins under-gravel shelters during winter and that in some cases an increase in shelters may result in denser populations of both these species. Work is now needed to test the effects of larger ranges of differences in size between the species and to understand how prior residence effects influence competition for shelter among sympatric populations of trout and salmon during natural seasonal changes in their habitat requirements.

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Using nonlinear hierarchical models for analyzing annulus-based size-at-age data

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Abstract: Size-at-age data for fish (derived from otoliths or other structures) are valuable but statistically messy. The data are typically serially correlated and unbalanced, with both time-independent and time-varying covariates. Appropriate growth models are typically nonlinear, with an unknown functional form. We recommend the use of nonlinear hierarchical models for the analysis of such data. We illustrate the use of these methods by applying the recently introduced SAS procedure NLMIXED (SAS Institute Inc., Cary, N.C.) to otolith-based estimated standard lengths of Utah chub (*Gila atraria*) collected in four locations with predators and four locations without predators.

Résumé : Les données de taille en fonction de l'âge chez les poissons (calculées à partir des otolithes ou d'autres structures) sont utiles, mais difficiles à traiter statistiquement. Ces données sont typiquement déséquilibrées et en corrélation séquentielle et elles possèdent des covariables dépendantes et indépendantes du temps. Les modèles de croissance qui leur conviennent sont généralement non linéaires et leur forme fonctionnelle est inconnue. Nous recommandons l'utilisation de modèles hiérarchiques non linéaires pour ces données. Nous en illustrons l'usage en appliquant la procédure récemment introduite de NLMIXED de SAS (SAS Institute Inc., Cary, N.C.) à des longueurs standard estimées à partir des otolithes chez des ménés de l'Utah (*Gila atraria*) provenant de quatre sites sans prédateurs et de quatre sites avec prédateurs.

[Traduit par la Rédaction]

Introduction

Many studies of ecology and evolution of fish populations use size measurements derived from scale and otolith annuli of captured fish (Osenberg et al. 1988; Jennings and Philipp 1992; Johnson and Belk 1999). Yearly estimates of body size-at-age (typically standard or total length) are back-calculated from annulus measurements (Tesch 1968; Campana 1990). A great deal of size-at-age information can potentially be obtained from relatively few fish, and the method has proven to be a powerful tool for characterizing growth patterns, comparing these patterns among populations, sexes, etc., and relating these patterns to environmental variables.

The main problem with analysis of annulus-based growth estimates is that the data are messy in a statistical sense. Because the captured fish are invariably of different ages, the series of size measurements are of different lengths. Growth patterns of fish, or other animals, are usually best described by nonlinear rather than linear functions of age (Chen et al. 1992). The back-calculated sizes within fish are serially correlated. An additional complication is that some explanatory variables or covariates apply to the fish (independent of their age, e.g., sex) and some vary over time within fish (e.g., age). Hence, data from annulus-based growth studies can be described as unbalanced, nonlinear repeated measures data, with both time-independent and time-varying covariates.

The statistical analysis of such data is further complicated by the fact that there is no consensus about which nonlinear function of age is appropriate for modeling fish growth (Chen et al. 1992). The choice of an appropriate nonlinear function often has to be made anew for each study because the functional form is dependent on such things as species, location, age-classes, and weight classes of the fish.

Several statistical approaches have been used in the analysis of annulus-based growth data. For example, to analyze differences among fish groups, one-way analysis of variance (ANOVA) and the one-way nonparametric Kruskal–Wallis procedure have been applied to size data separately for each age-class (Johnson et al. 1995; Belk 1998). Repeated measures ANOVA, treating age as a categorical variable, has been applied to complete series of size measurements for the fish (Jennings and Philipp 1992). Another approach has involved fitting a nonlinear, polynomial, or segmented linear model to the series of measurements for each fish and then comparing the parameter estimates among groups of fish using ANOVA or multivariate ANOVA (Johnson and Belk 1999). In a complicated analysis, Osenberg et al. (1988) computed growth rates as differences between annulus-based sizes for each size-class of each fish and then carried out factor analysis using the matrix of pairwise correlations of

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growth rates between size-classes. They examined the factor loadings for the various age-classes and interpreted them in terms of ecological factors.

Although valid, such approaches are incomplete, inefficient, and non-optimal. Some of these methods exclude data for certain fish or certain age-classes in order to deal with the unbalanced nature of the data. Some of them do not allow the use of nonlinear functions. Some ignore the serial correlations of the measurements. Potvin et al. (1990) emphasized the importance of using appropriate statistical tools for repeated measures data in ecological studies.

Another troubling issue in the statistical analysis of annulus-based growth data is that it is common for authors to apply several different statistical procedures to the same data set to answer different questions (Osenberg et al. 1988; Johnson and Belk 1999). Although not wrong, this practice can be confusing and inefficient. A more straightforward and productive general statistical paradigm would be first to select an appropriate model for the data using model fitting, model checking, and model comparison procedures and then to use the selected model to answer scientific questions by estimating appropriate functions of parameters of the selected model or by using the model to make predictions (McCullagh and Nelder 1989).

Fortunately, the last few years have seen great advances in statistical methodology for applying this general approach to exactly the type of data generated in annulus-based growth studies of fish. The methodology is associated with nonlinear mixed or hierarchical models. Reasonably accessible monographs have been written about the selection and analysis of nonlinear hierarchical models (Davidian and Giltinan 1995; Vonesh and Chinchilli 1996), and software has recently become available in major statistical packages to facilitate the use of these methods (SAS Institute Inc. 1999; Pinheiro and Bates 2000). McRoberts et al. (1998) recently recommended the use of nonlinear hierarchical models to investigate size-at-age relationships of animals and used these methods to analyze size-at-age data for black bears.

The methodology of nonlinear hierarchical models has apparently not been previously used for annulus-based fish growth data. The purpose of this paper is to describe the methodology and to apply it to data from a previously published fish growth study (Johnson and Belk 1999). In addition to demonstrating the methodology, a new nonlinear function for modeling fish growth is selected and new insights into Utah chub (*Gila atraria*) ecology are obtained.

Materials and methods

Nonlinear hierarchical models

Nonlinear hierarchical models are generalizations of both simple nonlinear statistical models and linear hierarchical models (Davidian and Giltinan 1995). The expected value of the response variable is modeled as a nonlinear function of known predictor variables, unknown fixed parameters, and unknown random quantities. The models are described as hierarchical because they accommodate data with a nested structure. For example, fish growth data consist of different numbers of repeated measurements for each of several individuals. Covariates and random effects are usually associated with all levels of the nested structure. The covariance structure of the random effects is specified as part of the model.

Sheiner et al. (1972) first recognized the need to account for variation within and among subjects in nonlinear models for pharmacokinetic data and proposed early linearized versions of nonlinear mixed models. Beal and Sheiner (1982, 1988) and Sheiner and Beal (1985) refined and extended the linearization methods. Racine-Poon (1985) used the expectation–maximization (EM) algorithm to estimate parameters of nonlinear mixed models. Lindstrom and Bates (1990) proposed a general nonlinear mixed model for longitudinal data. They used a variation of the linearization method together with Newton–Raphson iteration to estimate the mean and variance parameters. Vonesh and Carter (1992) considered nonlinear models for repeated measures with a variety of covariance structures and used a four-step generalized least squares method of estimation. Davidian and Giltinan (1995) reviewed, synthesized, and extended nonlinear hierarchical models and associated methods.

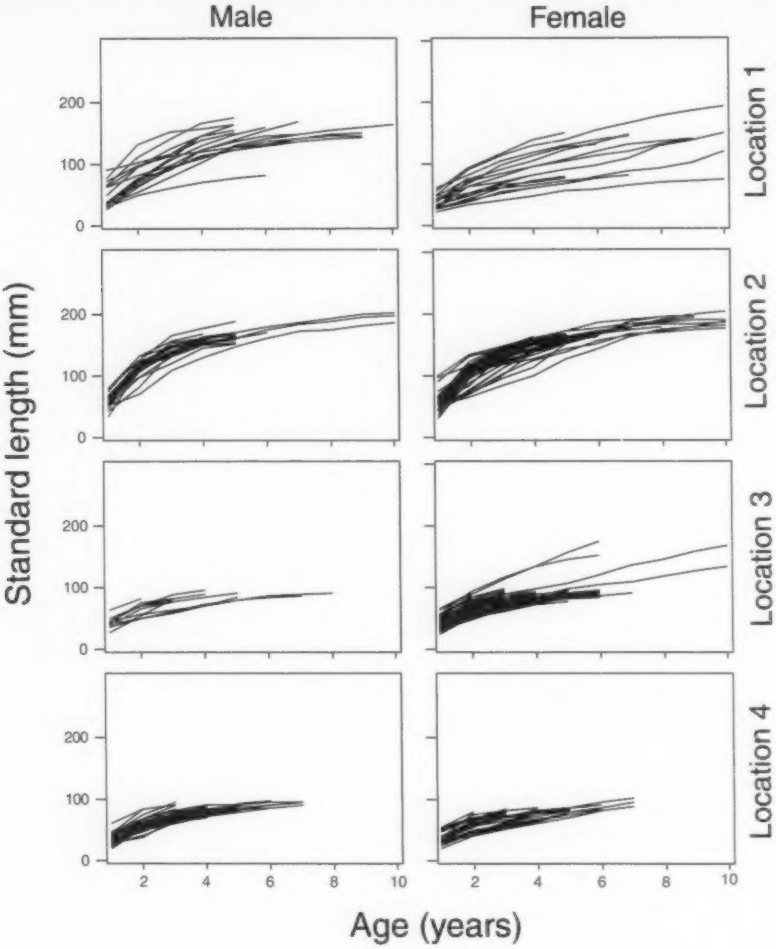
Nonlinear hierarchical models have received attention from researchers in the last decade because of the availability of high-speed computers and computationally intensive algorithms to find maximum likelihood estimates and their approximate covariance matrix. The likelihood function associated with a nonlinear hierarchical model can generally only be expressed as a product of several integrals involving conditional distributions, and therefore the iterative algorithm for maximizing the likelihood must include a numerical method for approximating these difficult integrals at each step. For low-dimensional integrals, Gaussian quadrature can be used. Pinheiro and Bates (1995) compared several different computational methods of finding maximum likelihood estimates for nonlinear mixed models (SAS Institute Inc. 1999).

Given the parameter estimates with their asymptotic covariance matrix, the delta method (Lehmann 1997) can be used to compute confidence intervals, prediction intervals, and hypothesis tests for nonlinear functions of the parameters.

A number of computer programs are currently available to carry out the extensive computations associated with nonlinear mixed-effect models. These include the NLME function of S-Plus (Pinheiro and Bates 2000), the SAS macro NLINMIX (Littell et al. 1996), and the SAS procedure NLMIXED (SAS Institute Inc. 1999). The NLMIXED procedure of SAS, the most recently developed of these programs, fits two-level nonlinear mixed effects models that are analogous to two-level linear random-coefficients models. It is unique in several ways. NLMIXED allows the conditional distribution of the response variable to follow the normal, binomial, or Poisson distribution. Several iterative optimization techniques are included in the SAS procedure as well as several integral approximation methods for evaluating the likelihood at each iteration. The ESTIMATE statement of NLMIXED can be used to estimate arbitrary linear or nonlinear functions of the fixed parameters along with their standard errors. The procedure also computes empirical Bayes estimates of random effects in the model.

To facilitate the selection of an appropriate nonlinear function on which to base the nonlinear hierarchical model, NLMIXED computes the Akaike information criterion (AIC; Akaike 1974) and the Bayesian information criterion (BIC;

Fig. 1. Otolith-based standard lengths of male and female Utah chub (*Gila atraria*) at four locations without predators. Each line represents a fish.



Schwarz 1978) for each fitted model. These criteria use the maximized likelihood penalized by the number of parameters in the model to indicate how well a model fits the data. Various candidate nonlinear functions can be used in developing nonlinear hierarchical models, and it makes sense to select the function producing the best value of the AIC or BIC (Littell et al. 1996).

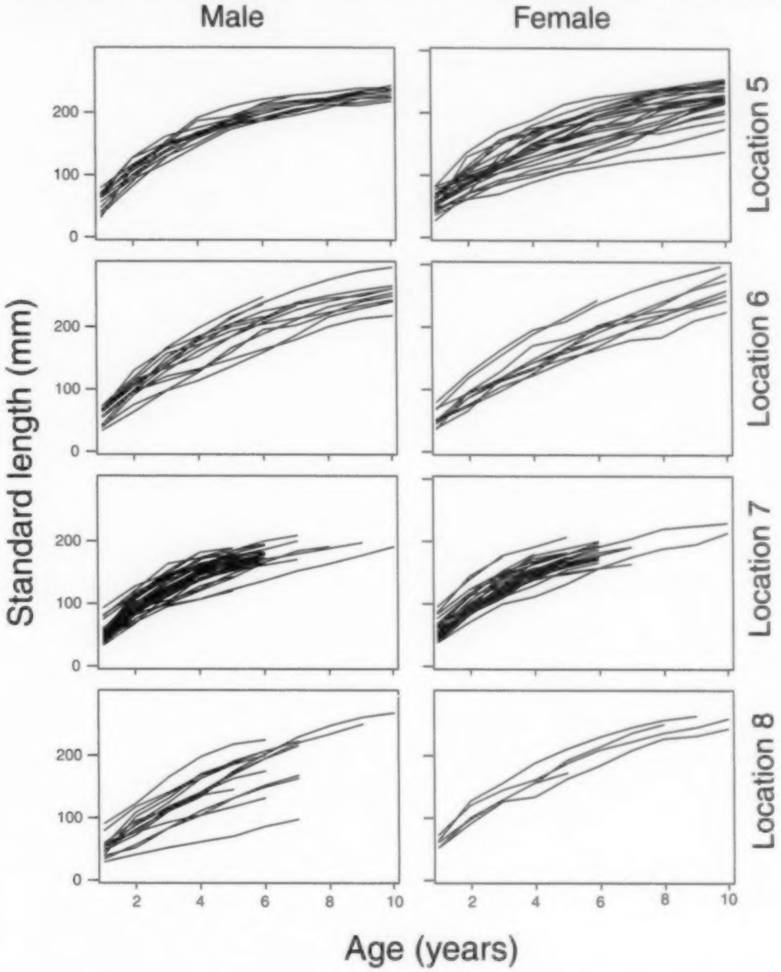
Utah chub data

Details of the fish collection and otolith measurement methods were given by Johnson and Belk (1999). Briefly, Utah chub were netted in eight locations in Wyoming and Utah, U.S.A. Sample sizes for adult Utah chub from the eight locations ranged from 19 to 67, and fish ages ranged from 1 to 29 years, but annulus measurements were made for the first 10 years only. Four of the locations were free of chub predators; predators were present in the other four locations. Back-calculated standard lengths for each age were generated using a modified Fraser–Lee formula (after Campana 1990). Profile plots for otolith-based standard lengths of the sampled fish by sex and location show that the growth curves all closely follow a nonsigmoid asymptotic shape, but with considerable variation in slopes and asymptotes among fish (Figs. 1, 2). The purpose of the study was to compare predator and nonpredator locations with regards to such life history traits as age at maturity, standard length at maturity, juvenile growth rate, and asymptotic standard length. Using three different statistical approaches, Johnson and Belk (1999) concluded that Utah chub in predator locations grew faster, reached a larger adult size, and matured at later ages and at larger sizes than did fish in nonpredator locations. This was consistently true for all locations except one nonpredator location (location 2), where the average growth rate was high compared with the other nonpredator locations. Johnson and Belk (1999) made no distinctions between sexes.

Statistical analysis of the Utah chub data

Our analysis followed the general statistical paradigm of model selection and checking followed by use of the se-

Fig. 2. Otolith-based standard lengths of male and female Utah chub (*Gila atraria*) at four locations with predators. Each line represents a fish.



lected model to answer scientific questions. For most nonlinear hierarchical modeling, the appropriate nonlinear function upon which the statistical model should be based is unknown and so the model selection stage involves two steps: selection of an appropriate nonlinear function and then selection of an appropriate statistical model based on the selected nonlinear function. We used the SAS procedure NLMIXED for all calculations associated with nonlinear hierarchical models.

Nonlinear function selection

An appropriate nonlinear function of age upon which to base the statistical model for Utah chub growth was selected by proposing a number of candidate growth functions with approximately the desired asymptotic shape, fitting a nonlinear hierarchical model based on each of these candidate functions to the Utah chub data, and calculating AIC and BIC for each model. To focus AIC and BIC on selection of the nonlinear function rather than on other aspects of the statistical model, nonlinear hierarchical models in this stage should include all or most of the potential statistical complexity. Also, the candidate nonlinear functions to be compared should be of comparable complexity.

As candidate nonlinear functions for use in developing a statistical model for the Utah chub data, four three-parameter functions discussed by Ratkowsky (1990) were used, all of which exhibit the general nonsigmoidal increasing shape displayed by the chub sizes (Figs. 1, 2) in at least part of their range:

(1) $\alpha - \beta\gamma^{x}$

(2) $\alpha/(1 + \beta x^{-\gamma})$

(3) $\alpha x^{\beta x^{-\gamma}}$

(4) $\alpha(1 - \beta^{-x})^{\gamma}$

In all of these functions, x is age and the parameters α, β, and γ are positive real numbers. Function 1 is the three-parameter von Bertalanffy growth function (von Bertalanffy

Table 1. Akaike information criterion (AIC) and Bayesian information criterion (BIC) values for the four candidate nonlinear functions describing growth of Utah chub.

	Information criterion	
Function	AIC	BIC
1	1369.3	1299.5
2	1459.9	1389.9
3	1501.1	1431.0
4	1434.1	1364.1

Note: Larger values indicate better fit.

Table 2. Likelihood ratio tests comparing six simpler models to the full statistical model.

Model	χ^2 value	df	p value
Additive	128	21	<0.0001
Constant α	213	15	<0.0001
Constant β	55	15	<0.0001
Constant γ	251	15	<0.0001
Nonrandom α	1352	2	<0.0001
Nonrandom β	705	2	<0.0001

1957), which has been commonly used historically for modeling growth of animals (McRoberts et al. 1998). However, there is no theoretical reason to believe that the von Bertalanffy growth function would be best or even appropriate for size-at-age relationships for fish (Chen et al. 1992).

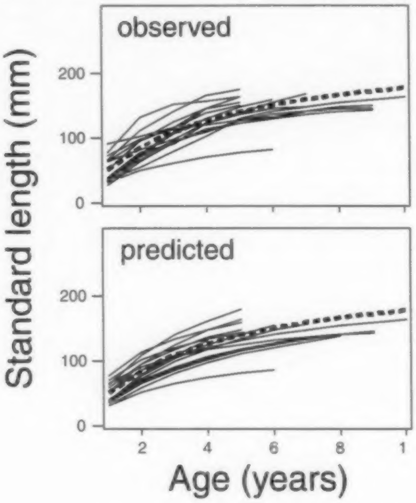
Relatively complex two-level nonlinear hierarchical statistical models were formulated based on each of these candidate functions. As an example, the statistical model based on function 3 was

$$y_{ijkl} = (\alpha_{ij} + a_{ijk})x_{ijkl}^{(\beta_{ij}+b_{ijk})x_{ijkl}^{-\gamma_{ij}}} + \varepsilon_{ijkl}$$

where y_{ijkl} is the lth standard length and x_{ijkl} the lth age of the kth Utah chub of sex i in location j. It was assumed that $a_{ijk} \sim n(0,\sigma_a^2)$, $b_{ijk} \sim n(0,\sigma_b^2)$, $\mathrm{cov}(a_{ijk}, b_{ijk}) = \sigma_{ab}$, and $\varepsilon_{ijkl} \sim n(0,\sigma_\varepsilon^2)$. This model thus included different fixed parameters α_{ij}, β_{ij}, and γ_{ij} for each sex–location combination, normally distributed and correlated random effects a_{ijk} and b_{ijk} for each fish within each sex–location combination, and a normally distributed random error associated with each standard length estimate for each fish. All of the models included age as a time-varying covariate, and sex and location as time-independent covariates. Hierarchical models based on the other candidate functions were formulated analogously.

Starting values for the parameter estimates were needed in order to fit these models and obtain maximum likelihood estimates of the parameters using NLMIXED. To find reasonable starting values, MAPLE V was used to plot the candidate functions for ranges of values of the parameters. Proc NLIN of SAS was then used to fit models based on the nonlinear functions to the data for each chub having at least four measurements. Means and variances of these parameter estimates were calculated and used as starting values for estimation of the fixed parameters and variance–covariance parameters of the random effects.

Fig. 3. Otolith-based standard lengths as well as model-based predicted standard lengths of male Utah chub (*Gila atraria*) at location 1. Each line represents a fish. The growth curve based on the estimated parameters for males in location 1 (broken line) is superimposed on the plots.



Statistical model selection

Once the nonlinear function of age was selected, NLMIXED was used to choose among several versions of the nonlinear hierarchical model of varying levels of statistical complexity. The full statistical model, also used in the preceding section, included fixed parameters α_{ij}, β_{ij}, and γ_{ij} for all sex–location combinations for a total of 48 fixed parameters. This model also involved the four variance–covariance parameters σ_a^2, σ_b^2, σ_{ab}, and σ_ε^2.

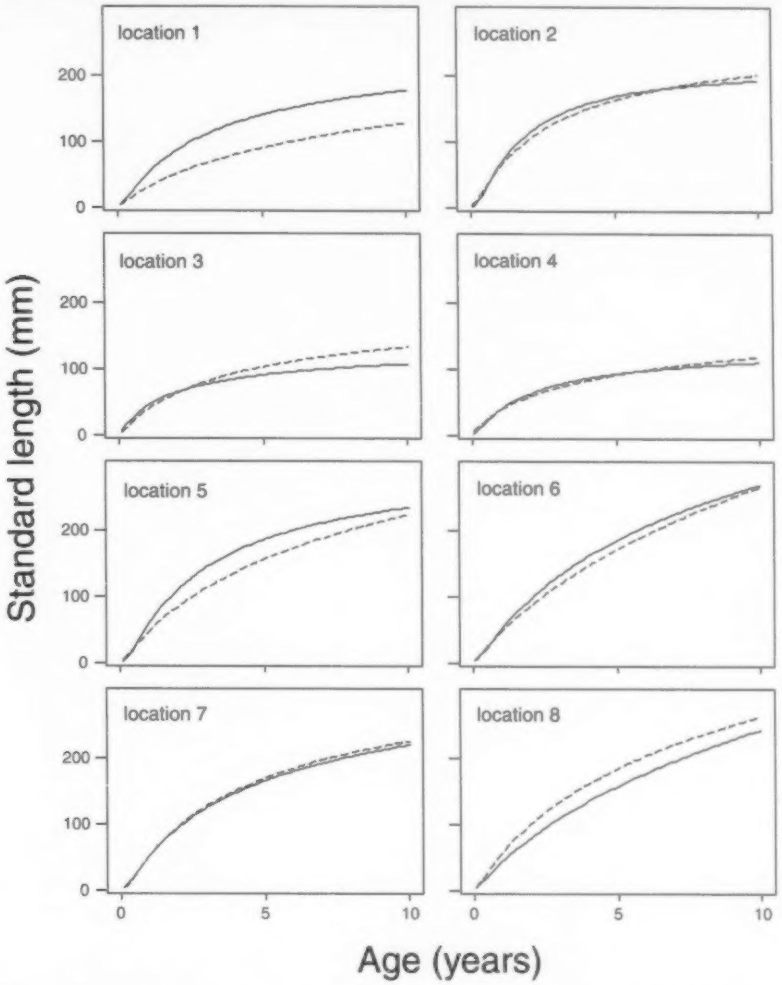
Six reduced statistical models were considered. The first of these, the additive model, had the same variance–covariance structure as the full model and thus involved the same four variance–covariance parameters. However, the parameters α_{ij}, β_{ij}, and γ_{ij} were constrained so that the effects of sex and location were additive. For example, α_{ij} was modeled as $\alpha + \theta_i + \phi_j$, and similar models were set up for β_{ij} and γ_{ij}. This model involved nine fixed parameters corresponding to each of α, β, and γ for a total of 27 fixed parameters.

The constant α model involved a single parameter α for all sex–location combinations but different parameters β_{ij} and γ_{ij} for each sex–location combination (33 fixed parameters, four variance–covariance parameters). The constant β and constant γ models were analogous to the constant α model.

The nonrandom α model had no random effects a_{ijk} (48 fixed parameters, two variance–covariance parameters). The nonrandom β model had no random effects b_{ijk} (48 fixed parameters, two variance–covariance parameters).

The goal in selection of a statistical model was to choose the most parsimonious well-fitting model. To choose between the full model and any of the reduced versions, likelihood ratio tests were carried out by calculating the difference between −2 times the log likelihood for each model

Fig. 4. Growth curves based on estimated parameters for Utah chub (*Gila atraria*) by location and sex: male, solid line; female, broken line.



and the full model. These test statistics are approximate chi-square random variables under the null hypothesis (i.e., if the reduced model is correct), with degrees of freedom calculated as the difference in the number of parameters for the two models (Lehmann 1997). The test is easily computed because NLMIXED automatically calculates the log likelihood for each model.

Using the selected model

After selecting and fitting an appropriate nonlinear statistical model, the model was used to carry out growth rate comparisons. For the particular nonlinear function selected for the Utah chub data, only one of the parameters had a natural biological interpretation. However, desired scientific questions could still be investigated using carefully chosen functions of the parameters. As an example, we considered the size at age 4 to be a meaningful indicator of the growth rate for each sex–location combination. This is a nonlinear function of the three parameters α_{ij}, β_{ij}, and γ_{ij} found by substituting $x = 4$ years, as well as sex and location information, into the selected nonlinear hierarchical model. This function was estimated by replacing the parameters in the function by their estimates, so that for function 3 the estimate of size at age 4 for a particular sex–location combination would be

$$\hat{\alpha}_{ij}(4^{\hat{\beta}_{ij}(4^{-\hat{\gamma}_{ij}})})$$

The approximate standard error of this estimate was obtained using the delta method (Lehman 1997), which involves a first-order Taylor series approximation evaluated at the estimated asymptotic variance–covariance matrix of the parameter estimates. These estimation and delta method calculations are carried out automatically by invoking the ESTIMATE statement of the NLMIXED procedure.

Using exactly the same general methods, more complicated functions of the parameters were also estimated along with their standard errors. Male–female differences in the average size at age 4 were estimated for each of the locations. For function 3, the average male–female difference in size at age 4 for location j would be estimated as

Table 3. Estimates (with standard errors, SE) of parameters of the statistical model based on function 3, as well as estimated standard length at age 4 (SE) for both sexes at every location for Utah chub (*Gila atraria*).

Sex	Predators	Location	$\hat{\alpha}_{ij}$	$\hat{\beta}_{ij}$	$\hat{\gamma}_{ij}$	Size at age 4 (mm)
M	No	1	49.1 (2.5)	0.923 (0.051)	0.217 (0.020)	126.8 (3.8)
		2	61.3 (2.6)	1.078 (0.047)	0.334 (0.014)	157.0 (3.1)
		3	43.6 (4.0)	0.634 (0.102)	0.210 (0.074)	84.2 (4.1)
		4	37.0 (2.2)	0.866 (0.067)	0.263 (0.034)	85.1 (2.9)
	Yes	5	59.0 (3.3)	1.075 (0.058)	0.252 (0.011)	168.8 (4.3)
		6	49.1 (3.0)	1.062 (0.047)	0.156 (0.012)	160.7 (5.3)
		7	53.0 (1.8)	0.981 (0.036)	0.199 (0.013)	148.7 (2.5)
		8	42.8 (2.7)	0.951 (0.045)	0.099 (0.018)	135.0 (5.3)
F	No	1	28.6 (2.3)	0.865 (0.052)	0.123 (0.025)	78.5 (5.1)
		2	58.2 (1.7)	0.949 (0.032)	0.243 (0.011)	148.8 (2.1)
		3	38.0 (1.6)	0.823 (0.040)	0.179 (0.022)	92.5 (2.2)
		4	36.6 (2.2)	0.715 (0.059)	0.147 (0.037)	82.2 (2.7)
	Yes	5	46.5 (2.3)	0.947 (0.034)	0.141 (0.010)	136.7 (4.0)
		6	45.7 (3.5)	0.954 (0.052)	0.094 (0.015)	145.8 (6.2)
		7	52.8 (2.1)	1.009 (0.039)	0.202 (0.014)	151.9 (3.1)
		8	55.9 (5.5)	0.934 (0.083)	0.141 (0.022)	162.1 (7.3)

Note: M, male; F, female.

Table 4. Estimated average differences (male minus female) in standard length at age 4 (in mm) with standard errors (SE) of Utah chub (*Gila atraria*) at each location.

Location	Predators	Difference	SE	t	p value
1	No	48.2	6.3	7.66	<0.0001
2		8.2	3.7	2.18	0.0296
3		−8.3	4.7	−1.78	0.0751
4		2.9	4.0	0.73	0.4639
5	Yes	32.1	5.9	5.46	<0.0001
6		14.9	8.1	1.84	0.0660
7		−3.1	4.0	−0.79	0.4311
8		−27.1	9.0	−3.00	0.0029

$$\hat{\alpha}_{1j}(4^{\hat{\beta}_{1j}(4^{-\hat{\gamma}_{1j}})}) - \hat{\alpha}_{2j}(4^{\hat{\beta}_{2j}(4^{-\hat{\gamma}_{2j}})})$$

Similarly, average predator–nonpredator location differences were estimated overall and for each sex. Because locations were treated here as fixed effects, our inferences about the difference between predator and nonpredator locations applies to these particular locations, not to the general populations of such locations.

Other possibly interesting indicators of growth patterns are the age at which a specified size is achieved, the derivative of the size-at-age function at a specified size or age, the size difference between two specified ages, and the age at which the derivative of the size-at-age function is maximized. If these functions of the parameters of the model exist, they can similarly be estimated and compared statistically.

Results

Nonlinear function selection

Difficulties were encountered when we attempted to fit the hierarchical model based on the von Bertalanffy growth function to the data. Regardless of starting values, the procedure would not converge. Through a process of trial and error, including fitting the model to each group separately, it was discovered that if the males and females for location 3 and the males for location 4 were left out, the hierarchical model based on the von Bertalanffy growth function could be fit. Hence, the decision was made to eliminate locations 3 and 4 when fitting all of the nonlinear models to the data so the AIC and BIC values would be comparable among the nonlinear models. The AIC and BIC values both indicated that function 3 should be selected as the nonlinear function upon which to base the statistical model (Table 1). Interestingly, the model based on the von Bertalanffy growth function had the worst values of AIC and BIC.

Statistical model selection

Likelihood ratio tests based on the complete data set (including locations 3 and 4) indicated that the full statistical model based on function 3 (with different values α_{ij}, β_{ij}, and γ_{ij} for all sex–location combinations) was significantly better than the additive, constant α, constant β, constant γ, nonrandom α, and nonrandom β models (Table 2). Estimates and standard errors of the 48 fixed parameters of the nonlinear hierarchical model were computed by NLMIXED (Table 3), as were estimates of the variance–covariance parameters σ_a^2, σ_b^2, σ_{ab}, and σ_ε^2. These latter estimates were 150.0, 0.024, −0.017, and 40.0, respectively. The predicted growth curves agreed extremely well with the observed data (Fig. 3), and the estimated growth curves for all sex–location groups exhibited interesting differences (Fig. 4).

Growth rate comparisons

Using the full model, the average size at age 4 was estimated for each sex–location group (Table 3). Male–female differences in the average size at age 4 varied among locations (Table 4). In three of the locations, two without predators and one with predators, males were significantly larger than females (using p value ≤ 0.05). Females were significantly larger at age 4 than males in one predator location. In the remaining four locations, the male–female differences in size at age 4 were not significant.

In general, fish at age 4 were significantly larger in locations with predators than in locations with no predators. Male chubs were, on average, 11.8 mm larger in predator locations than in nonpredator locations. The standard error (SE) of this difference was 5.2 mm. Female chubs were estimated to be 25.0 mm (SE = 5.8 mm) larger in predator than in nonpredator locations. Location 2 was the only nonpredator location in which the estimated size at age 4 was comparable to values for predator locations.

Discussion

Comparison to previous analyses of the Utah chub data

Analysis of the Utah chub data based on a nonlinear hierarchical model gave similar conclusions to those obtained by Johnson and Belk (1999). Both analyses revealed significant differences in the growth patterns of Utah chub living in the presence of predators versus those living in locations without predators. Both analyses also indicated that location 2, which had no predators, had growth patterns similar to predator locations. Agreement between the current and previous results is expected because the previous approach (of fitting a model—von Bertalanffy growth model or two-phase regression—to the data for each fish and then applying ANOVA to the parameter estimates for the fish) is similar to a nonlinear hierarchical model. The main difference is that the former procedure is ad hoc and inefficient. The methodology of nonlinear hierarchical models combines the data from all of the fish optimally and produces explicit estimates of relevant variance–covariance parameters.

Our analysis selected a nonlinear function (function 3) of age that apparently has not been used previously in fish growth studies. The use of models based on function 3 in other size-at-age studies should be considered, at least if theoretical considerations do not suggest the use of any other function. Neither β nor γ has a natural biological interpretation, but α is the size at age 1. However, as noted above, relevant biological variables can be defined as functions of fixed parameters of the model and then estimated and compared statistically.

Analysis based on the nonlinear hierarchical model revealed that the relationship between growth patterns, sexes, and locations is very complicated for the Utah chub. The presence of predators was associated with higher growth rates in general, but the effect was quite variable. Apparently, other environmental factors also affect growth rates. Additionally, there was a significant male–female difference in growth rates at some locations, but this effect was not consistently associated with the presence or absence of predators. The results could suggest interesting evolutionary and ecological hypotheses.

Nonlinear hierarchical models for annulus-based size-at-age data

Using nonlinear hierarchical models in the analysis of the annulus-based size-at-age data for the Utah chub was labor-intensive. We had to carefully study the user instructions for the NLMIXED procedure, including numerical options and optional statements such as those giving starting values and bounds for the parameters estimates. Trial and error were required to obtain appropriate starting values and successful numerical methods. Even after resolving these computational problems, several model-fitting runs were needed to compare various candidate nonlinear functions and statistical models and to estimate and compare the desired functions of the parameters. In many cases, it would be advisable to obtain expert statistical help when analyzing size-at-age data with nonlinear hierarchical models.

Nonetheless, the approach had several advantages. Nonlinear hierarchical models easily handled unbalanced and incomplete repeated measures data. Models based on alternate nonlinear functions were readily compared, and a statistical model of an appropriate degree of complexity was objectively selected. Estimates of variance and covariance components were used in calculations of tests and confidence intervals for interesting functions of the parameters.

The availability of software and technology for nonlinear hierarchical models allows annulus-based size-at-age data to be analyzed using the general statistical approach of model selection followed by use of the selected model to answer scientific questions. This approach simplifies the statistical analysis, leads to valid and defensible statistical results, and simplifies the reporting of methods and results. We recommend the general use of these methods for analyzing annulus-based size-at-age data.

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Comparison of equilibrium and nonequilibrium estimators for the generalized production model

Erik H. Williams and Michael H. Prager

Abstract: Parameter estimation for the logistic (Schaefer) production model commonly uses an observation-error estimator, here termed the NM estimator, combining nonlinear-function minimization and forward projection of estimated population state. Although the NM estimator can be used to fit the generalized (Pella–Tomlinson) production model, an equilibrium approximation method (EA) is often used, despite calls in the literature to abandon equilibrium estimators. We examined relative merits of NM and EA estimators for the generalized model by fitting 48 000 simulated data sets describing five basic stock trajectories and widely varying population characteristics. Simulated populations followed the generalized production model exactly but were observed with random error. Both estimators were used on each data set to estimate several quantities of management interest. The estimates from NM were usually more accurate and precise than EA estimates, and overall, NM outperformed EA. This is the most comprehensive study of the question to date—the first to examine the generalized production model—and it demonstrates clearly why equilibrium estimators should be abandoned. Although valuable when introduced, they are no longer computationally necessary, they are no less demanding of data, and their performance is poor.

Résumé : L'estimation des paramètres du modèle logistique de production (Schaefer) fait ordinairement intervenir un estimateur de l'erreur d'observation, l'estimateur NM, qui combine une minimisation d'une fonction non linéaire et une projection vers le futur d'une estimation de l'état de la population. Bien que l'estimateur NM puisse être utilisé pour ajuster la forme généralisée du modèle de production (Pella–Tomlinson), on emploie souvent une méthode d'approximation d'équilibre (EA), même si de nombreux auteurs déconseillent cette utilisation. Nous avons comparé le mérite des estimateurs NM et EA utilisés dans le modèle généralisé en ajustant 48 000 ensembles de données simulées qui décrivent cinq évolutions de stocks fondamentales et des caractéristiques démographiques très variées. Les populations simulées suivent le modèle avec précision, mais elles affichent des erreurs aléatoires. Les deux estimateurs ont été employés sur chaque ensemble de données pour calculer plusieurs valeurs d'intérêt pour la gestion. Les estimations faites à partir de NM sont généralement plus précises et, tout compte fait, l'estimateur NM fonctionne beaucoup mieux que EA. Notre étude de ce problème est la plus vaste à ce jour et elle démontre clairement pourquoi les estimateurs EA doivent être abandonnés. Bien qu'utiles lors de leur introduction, ces estimateurs ne sont plus nécessaires, étant donné les ressources informatiques actuellement disponibles. De plus, ils exigent autant de données et leur performance est faible.

[Traduit par la Rédaction]

Introduction

Simple surplus production models (those without age structure) continue to prove useful in stock assessment, both when age data are lacking or unreliable and to provide direct estimates of maximum sustainable yield (MSY), an important measure of potential stock productivity (e.g., Zhang et al. 1991). As with all models, care in implementation and choice of statistical methods can be as important as model definition itself (Xiao 1997, 2000). For example, Fox (1971) described the importance of the form of objective function in fitting the generalized production model of Pella and Tomlinson (1969); Schnute (1989) demonstrated the importance of choice of conditioning (the assumption on which observed quantity, yield or relative abundance, should be assumed known without error) in fitting a logistic surplus-production model (Schaefer 1954, 1957), which he used to exemplify a relatively simple fishery model. Ludwig et al. (1988) and Polacheck et al. (1993) compared observation-error and process-error estimators, among other issues of statistical model structure.

Current practice in parameter estimation for the simplest production model, the logistic (Graham–Schaefer) model (Graham 1935; Schaefer 1954, 1957), usually takes the form of an observation-error estimator comprising nonlinear-function minimization and forward projection of estimated population state. That method of parameter estimation, here denoted NM (for nonlinear minimization), was introduced to fishery science by Pella (1967) and Pella and Tomlinson (1969) and was modified by Prager (1994) to incorporate a logarithmic objective function (reflecting assumed lognormal errors in the observed data) and conditioning on yield, in keeping with findings of Fox (1971) and Schnute (1989). The method

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is also described by Hilborn and Walters (1992), Quinn and Deriso (1999), and Haddon (2001). Similar methods were introduced for catch-at-age models by Methot (1989) and, because of their flexibility, are increasingly used in fishery science for both simple and complex models.

The NM estimator avoids the so-called "equilibrium assumption", in which observed yields are assumed to represent equilibrium yields, an assumption usually made about data on fishing effort averaged over several years; the averaging is said to approximate equilibrium conditions (Gulland 1961; Fox 1975*a*). Equilibrium estimators make short-cut methods of parameter estimation possible—and that was of great importance before the wide availability of small, powerful computers—but they lack a solid theoretical foundation.

Several studies of the logistic production model have found that significant biases can occur when using equilibrium estimators. Sissenwine (1978) pointed out that equilibrium methods can provide an answer, if a possibly misleading one, when data do not support estimation by nonequilibrium methods. That occurs not because equilibrium estimators require less extensive or less precise data (which they do not), but because they are generally linear approximations, and thus least-squares parameter estimates can be computed easily. In contrast, most nonequilibrium estimators rely on nonlinear optimization techniques that may fail to converge when the data conflict with the model.

Mohn (1980) evaluated equilibrium estimators on a simulated stock trajectory of increasing effort, stock collapse, and recovery. Results from simulated data with two levels of population productivity, four levels of noise, and variations of several other parameters were reported, with 50 replications of each. Mohn (1980) found that equilibrium estimators in general overestimated MSY and f_{MSY}, the rate of fishing effort rate at which MSY can be attained. By fitting sections of the population trajectories, Mohn (1980) found that the trend in population level affected the degree of bias in the equilibrium estimates. Less bias in f_{MSY} but greater overestimation of MSY occurred from an increasing trend in population levels than from a decreasing trend (Mohn 1980).

Roff and Fairbairn (1980) noted that bias in the equilibrium method of parameter estimation is not alleviated by increasing the number of years used in the effort averaging and argued that, on statistical grounds alone, the equilibrium method cannot be justified.

The most extensive previous comparison of equilibrium and nonequilibrium methods was by Polacheck et al. (1993). Using population parameters from two fits to data on three real fish stocks, the authors simulated 500 data sets for each set of parameters. Those data were then fit with three estimators for the logistic production model: an observation-error estimator similar to the NM estimator used here, a process-error estimator, and an effort-averaging method similar to the equilibrium-approximation (EA) estimator used here. Of the three, the observation-error estimator gave the least biased and most precise estimates of MSY and f_{MSY}. The effort-averaging estimator produced seemingly reasonable estimates of MSY and f_{MSY} that were nonetheless positively biased. The performance of the equilibrium estimator led Polacheck et al. (1993) to recommend "that effort-averaging (equilibrium) methods be abandoned". As will be seen, our study is more exhaustive than that of Polacheck et al. (1993), and unlike that study, we examine estimators for the generalized production model.

Current practice in parameter estimation for the generalized production model (Pella and Tomlinson 1969) is more varied than for the logistic model. Despite the introduction of nonequilibrium fitting methods over 30 years ago by Pella and Tomlinson (1969), the equilibrium-approximation (here, EA) estimation method due to Fox (1975*a*) after Gulland (1961) is still in current use, e.g., for assessments of some tuna species (ICCAT 2000), perhaps because fitting the generalized model without the approximation can be difficult. That difficulty stems from at least three causes: model structure, data insufficiency, and implementation quality. Fletcher (1978) restructured the model equations to give the parameters understandable meanings and reduce correlation among parameters; nonetheless, the model shape can be difficult to estimate. By model shape, we mean the degree and direction of asymmetry in the production curve (Fig. 1).

That many data sets possess insufficient information to specify model shape unambiguously was noted by Fletcher (1978) and Hilborn and Walters (1992), among others. Prager (2002) confirmed Fletcher's (1978) observation that the problem of data insufficiency can be accentuated by anomalous observations in the data set.

Implementation of the generalized model is not trivial: if a continuous-time formulation is used, numerical approximations are required to integrate the catch equation and to obtain its solution when conditioning on yield. Even when using a discrete-time model, the objective function can be characterized by many local minima. Thus, the computational simplicity of the EA estimator is appealing. Given this situation, a natural topic of inquiry is the comparative performance of the two methods, EA and NM, on varied data sets. That topic is the main subject of this study.

Methods

Overview

We employed computer simulation to compare the NM and EA estimators for the generalized production model, using methods (Fig. 2) described in detail below. The initial step in the study was generating numerous data sets representing simulated fished populations. Each simulated population followed the dynamics of the generalized production model exactly, a choice made not because real fish populations follow any model exactly, but because a logical start in evaluating estimators is checking their performance on data that meet their most important assumption (model structure). Simulated populations were "observed" by recording total stock biomass and removals (in arbitrary units, here designated kg) taken each time period (designated years). Simulated observation error was added to each year's data, the resulting data sets were fit using both estimators, and the resulting estimates of management-related quantities were compared (graphically and numerically) to the true underlying values, known from the simulations.

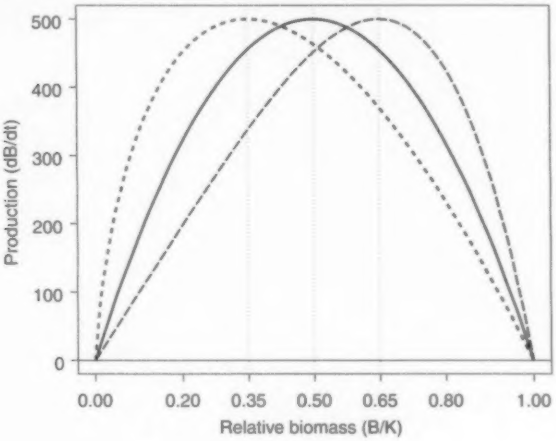


Fig. 1. Production curves characteristic of simulated populations used in study. Curves correspond to $\phi = 0.35$ (short-dashed line), 0.50 (solid line), and 0.65 (long-dashed line).

Simulation of data

A simulated population following production-model dynamics is characterized by three basic quantities; in the parameterization chosen (Fletcher 1978; Prager 2002), these are maximum sustainable yield, MSY; carrying capacity, K; and model shape, ϕ. We define $\phi = B_{\mathrm{MSY}}/K$, where B_{MSY} is the population biomass at which maximum surplus production occurs and $0 < \phi < 1$ is a dimensionless parameter that defines the shape of the production curve, i.e., the curve of surplus production against population biomass (Fig. 1). To generate a specific population trajectory, it is also necessary to specify the biomass in the first year, B_1, and the catchability coefficient, q, that relates the simulated abundance index I_t to the actual size of the population B_t by $I_t = qB_t$ (where subscript t indicates time in steps of 1 year). Here, each population was characterized by K = 1 000 000 kg and its abundance index by $q = 1$. We varied three quasi-biological characteristics of these simulated populations: general shape of the historical biomass trajectory, shape of the production curve, and value of F_{MSY}, the fishing mortality rate associated with MSY (Fig. 2). Because we set F_{MSY} and ϕ for each simulated population, MSY was not set directly, but rather by the relationship MSY = $\phi K F_{\mathrm{MSY}}$. Starting biomass B_1 was set for each simulated population based on the shape of the biomass time trajectory and the value of ϕ, as described below. Each simulated data set was 40 years long.

Populations were projected using the reformulated Pella–Tomlinson model of Fletcher (1978). Under that model, the biomass rate of change is given by

$$\text{(1)} \qquad \frac{\mathrm{d}B_t}{\mathrm{d}t} = \gamma \mathrm{MSY}\frac{B_t}{K} - \gamma \mathrm{MSY}\left(\frac{B_t}{K}\right)^n - q f_t B_t$$

where the third term describes removals by fishing and the notational quantity γ is given by

$$\gamma = \frac{n^{n/(n-1)}}{n-1}$$

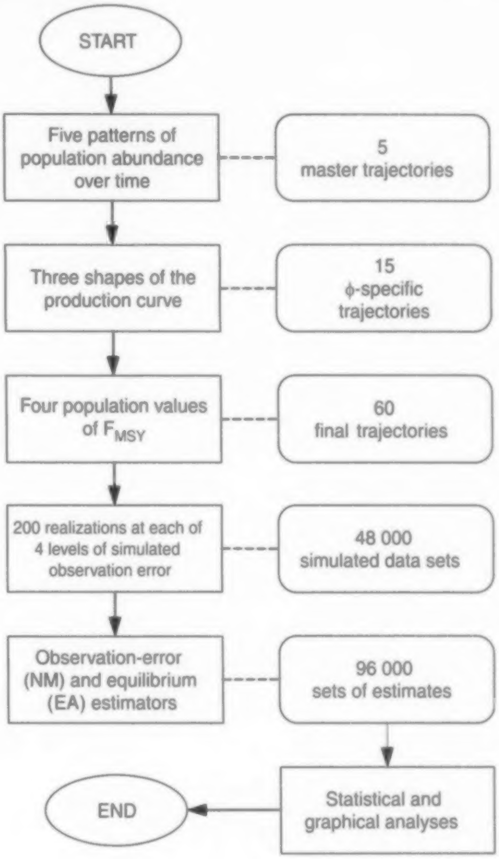

START
Five patterns of population abundance over time
5 master trajectories
Three shapes of the production curve
15 ϕ-specific trajectories
Four population values of F_MSY
60 final trajectories
200 realizations at each of 4 levels of simulated observation error
48 000 simulated data sets
Observation-error (NM) and equilibrium (EA) estimators
96 000 sets of estimates
Statistical and graphical analyses
END

Fig. 2. Schematic of study: generation of simulated data, fitting, and evaluation.

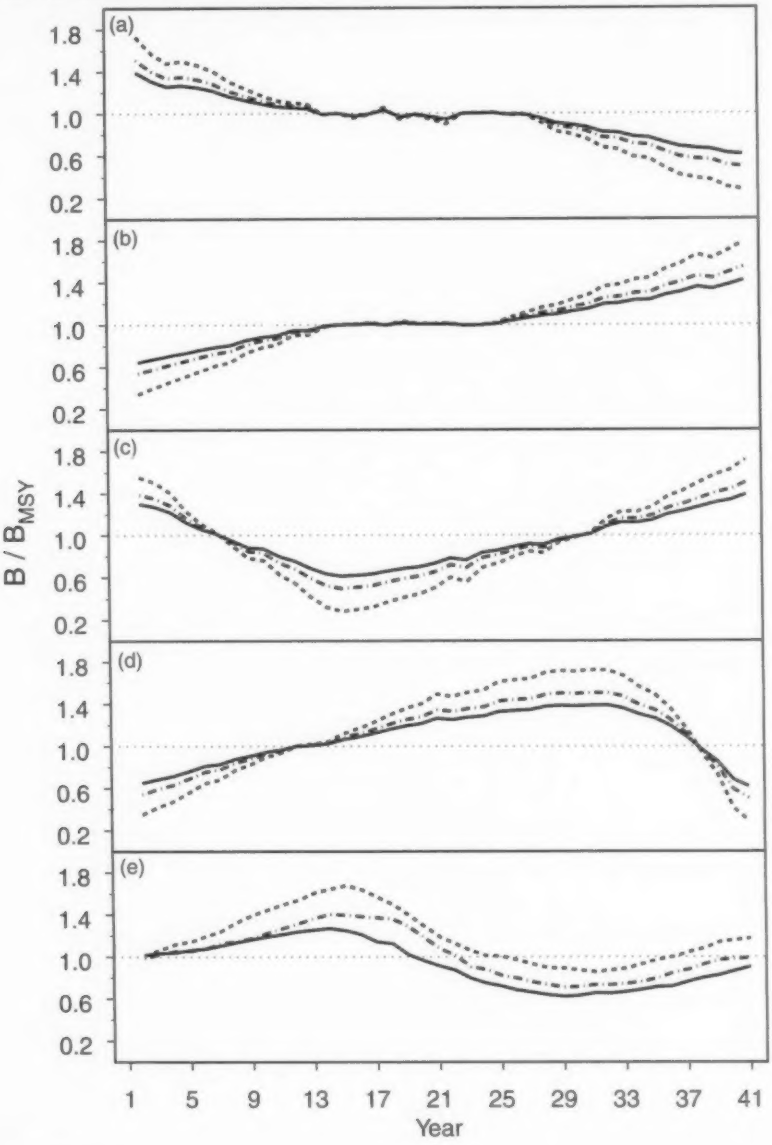
In eq. 1, shape of the production curve is determined by the exponent n, but in this paper, we describe model shape by the dimensionless parameter ϕ. The two are related by $\phi = B_{\mathrm{MSY}}/K = (1/n)^{1/(n-1)}$ (Fletcher 1978).

Starting from the simulated value of B_1, each simulated population was projected forward by numerical integration of eq. 1, using 90 time steps per year, which provides a close approximation to a continuous-time model. Simulated annual catches and start-of-year biomass levels were recorded for fitting.

Master trajectories

The sequence of data generation and analysis was simple, yet the number of factors considered and desire for sufficient replication led to a large number of simulated data sets (Fig. 2). To serve as the basis of all simulations, five master biomass trajectories were generated as arbitrarily scaled time series of annual biomass, B_t/B_{MSY}. The five master trajectories can be characterized as decreasing (trajectory 1), increasing (trajectory 2), concave (trajectory 3), convex (trajectory 4), and complex (trajectory 5); together, they represent a wide range of possible stock dynamics (illustrated after scaling to B_{MSY} by the dash–dot lines in Fig. 3).

Fig. 3. Simulated population trajectories used to study competing estimators for generalized production model. Curves correspond to ϕ = 0.35 (short-dashed line), 0.50 (dash–dot line), and 0.65 (solid line).



Shapes of the production curve

The generalized production model is used to accommodate possible skewness of the production curve. Each of the five master biomass trajectories was transformed to each of three shapes of the production curve at which data were simulated, $\phi = \{0.35, 0.5, 0.65\}$ (Fig. 1), to result in 15 ϕ-specific trajectories (Figs. 2, 3). The value $\phi = 0.35$ is very close to the shape of the Fox (1970) exponential yield model, for which $\phi = 1/e \approx 0.37$. The central value $\phi = 0.5$ defines the logistic (Schaefer) model, and $\phi = 0.65$ is symmetrical with $\phi = 0.35$.

With K held constant (as it was in our simulations), each model shape corresponds to a different value of B_{MSY}, because $B_{\mathrm{MSY}} = \phi K$. Transformations made for ϕ were as follows. Master biomass trajectory shapes 1–4 were simply translated up (for $\phi = 0.35$) or down (for $\phi = 0.65$) on the biomass axis so that the corresponding ϕ-specific trajectories passed through B_{MSY} at the same time (Figs. 3*a*–3*d*). The curves in those panels appear to have different shapes for each value of ϕ, because they are shown on a metric of B/B_{MSY}. If plotted in a metric of absolute biomass (or B/K), the three curves within each panel would be parallel.

The adjustments made to trajectory 5 were slightly different. In that case, it proved necessary to further adjust the trajectories to be realizable across a variety of population values of F_{MSY}, as described immediately below. The three trajectories were translated in the same manner as described for trajectories 1–4, and then heuristic adjustments were made to arrive at realizable trajectories (Fig. 3*e*).

Population values of F_{MSY} and simulation of yield

Four levels of F_{MSY} (0.1, 0.2, 0.4, and 0.8 per year) were chosen to represent a wide range of population productivity. These were applied to the 15 ϕ-specific trajectories to generate 60 final trajectories (Fig. 2). In generating final trajectories derived from master trajectories 1–4, biomass time series were the same for all values of F_{MSY}, but corresponding trajectories of F_t differed. Because $MSY = \phi K F_{MSY}$, the different values of F_{MSY} imply corresponding values of MSY.

Annual fishing mortality (F_t) values corresponding to each of the 60 final trajectories were computed using an iterative algorithm that searched for suitable values under production-model dynamics. The annual values of fishing mortality rate (and the value of B_1/K) were selected to produce the desired final trajectory under the simulated population's characteristic values of ϕ and F_{MSY}. This procedure also provided the simulated yield (catch) for each year.

Observation error

The final step in data simulation was construction of simulated abundance indices by adding lognormal observation error to the simulated biomass in each final trajectory. (Process error was not considered in this study.) In doing this, we generated 800 simulated data sets from each final trajectory (Fig. 2) by adding different random errors 200 times each at four levels of observation error, i.e., at coefficients of variation (CVs) of 2, 5, 15, and 25%. We consider the highest two values the most realistic; the lower values were included to observe results over a wider range. The result was the 48 000 simulated data sets to be used in the fitting part of the study.

Estimators

To implement the EA estimator, we used the Fortran computer program PRODFIT of Fox (1975*b*), slightly modified by us. Modifications included correction of a few obvious coding errors and limiting Fox's user-specified smoothing parameter to a single value, rather than a value for each year; the latter is apparently standard practice among some users of PRODFIT. We also modified the output recorded from PRODFIT to facilitate comparisons between estimators.

Parameters are estimated in the PRODFIT program under a least-squares objective function using a simple pattern-search optimization routine. Before estimation, the program adjusts the recorded annual fishing effort rates in an attempt to approximate equilibrium conditions. This is done by computing annual weighted averages of the fishing effort rate for *k* years, with *k* being the number of year-classes "making a significant contribution to the catch" (Fox 1975*a*, 1975*b*). The parameter *k* must be specified by the user: we set *k* to the number of year-classes necessary to account for >80% of the catch with a constant total mortality rate set at $Z = 2F_{MSY}$, which corresponds to 9, 5, 3, and 2 year-classes for F_{MSY} = 0.1, 0.2, 0.4, and 0.8 $year^{-1}$, respectively.

On certain data sets, PRODFIT results indicate a model termed the "asymptotic model" by the program and by Fox (1975*a*), but otherwise not documented. This appears to be a model in which ϕ is asymptotically close to zero; under such a model, maximum sustainable yield would be taken at fishing mortality rates approaching infinity and population sizes approaching zero. Such a model implies that for all $B_t > 0$, $B_t/B_{MSY} = \infty$ and for all $F_t < \infty$, $F/F_{MSY} = 0$. Such results were considered completely unrealistic and thus considered estimation failures.

To implement the NM method, we used a modified version of the ASPIC software of Prager (1995), which employs a nonequilibrium approach to fitting the parameters of the generalized production model. The method includes estimation conditional on observed catch, assuming lognormal observation error in the abundance index. In the ASPIC program, parameters are estimated by minimization of a lognormal objective function using the simplex or "polytope" algorithm of Nelder and Mead (1965). Numerous restarts of the algorithm are used to avoid local minima. Parameters are MSY (maximum sustainable yield), *K* (carrying capacity), *q* (catchability coefficient), B_1 (biomass at the start of the first year), and ϕ (shape of the production curve). The major modification to the program described by Prager (1995) was the addition of an algorithm to integrate the generalized model numerically so that its parameters could be estimated. Starting guesses for parameters were obtained from a fit of the logistic production model ($\phi = 0.5$). The fitting procedure is described in more detail in Prager (1994) and Prager (2002).

The GENPROD computer program of Pella and Tomlinson (1969) also provides nonequilibrium estimates for the generalized production model. Our estimation approach differs from that of Pella and Tomlinson (1969) mainly in (*i*) conditioning on yield, rather than fishing effort, (*ii*) finer resolution in the numerical integration, (*iii*) use of a logarithmic objective function, (*iv*) use of Fletcher's (1978) restructuring of the generalized model, (*v*) characterization of model shape by the ratio $\phi = B_{MSY}/K$, rather than the exponent, (*vi*) use of a different optimizer (Nelder and Mead 1965), and (*vii*) direct estimation of the shape parameter, rather than use of a coarse grid of values. Many of these changes were made possible by the enormous improvements in computers since the work of Pella and Tomlinson (1969).

Results

Results presented here focus on estimation of four quantities of management interest: $B_./B_{MSY}$, $F_./F_{MSY}$, MSY, and ϕ. We use the notation $B_./B_{MSY}$ to mean the biomass at the end of the final year, relative to B_{MSY}, and $F_./F_{MSY}$ to mean the fishing mortality rate during the final year, relative to F_{MSY}. Box–whisker plots are used to allow visual assessment of accuracy and precision of estimates. For further summarization, results are presented as median proportional unsigned error (MPUE, defined below) in comparable groups of estimates.

Realized values of the status indicators ($B_./B_{MSY}$ and $F_./F_{MSY}$) for each simulated population trajectory are given (Table 1) to aid interpretation of estimation results. The values vary by trajectory, and within trajectory, the true value of $B_./B_{MSY}$ varies by model shape ϕ, whereas the true value of $F_./F_{MSY}$ varies by ϕ and population value of F_{MSY}.

Graphical presentation of results

Similar graphical presentations are used for results in each

Table 1. Underlying values of management quantities in simulated populations following generalized production model.

Simulated trajectory	$B./B_{\text{MSY}}$			$F./F_{\text{MSY}}$		
	$\phi = 0.35$	$\phi = 0.5$	$\phi = 0.65$	$\phi = 0.35$	$\phi = 0.5$	$\phi = 0.65$
1[a]	0.28	0.50	0.61	3.13–2.42	1.83–1.54	1.44–1.26
2[a]	1.78	1.54	1.42	0.02–0.40	0.13–0.43	0.07–0.31
3[a]	1.72	1.50	1.45	0.001–0.42	0.07–0.47	0.04–0.23
4[b]	0.29	0.50	0.62	2.59–5.70	1.66–3.06	1.35–2.26
5[a]	1.18	0.99	0.91	0.72–0.83	0.90–1.00	0.68–1.04

Note: Range in population F_{MSY} was 0.1–0.8 year^{-1}. Definitions: B_{MSY}, biomass at which maximum sustainable yield can be obtained; F_{MSY}, corresponding instantaneous rate of fishing mortality; $B.$, biomass at end of final year of simulation; $F.$, fishing mortality rate during final year of simulation; ϕ, shape parameter of generalized model expressed as ratio of B_{MSY} to carrying capacity K.

[a] In ranges shown, higher $F./F_{\text{MSY}}$ corresponds to population with higher F_{MSY}.

[b] In ranges shown, higher $F./F_{\text{MSY}}$ corresponds to population with lower F_{MSY}.

quantity for each trajectory (Figs. 4–13); that presentation is explained in detail here. A set of three panels displays estimation results for a single quantity for a single trajectory (e.g., Figs. 4*a*–4*c* for estimates of $B./B_{\text{MSY}}$ from trajectory 1). The top panel (*a* or *d*) of the three shows results for $\phi = 0.65$; the center panel (*b* or *e*), for $\phi = 0.5$; the bottom panel (*c* or *f*), for $\phi = 0.35$. Within each panel, estimates from the NM estimator are in the upper row and estimates from the EA estimator are in the lower row. Within each row are three subpanels with results from the four cases of F_{MSY}. The subpanel labeled "Low" corresponds to $F_{\text{MSY}} = 0.1$, "Med" (medium) to aggregated results from $F_{\text{MSY}} = 0.2$ and 0.4, and "High" to $F_{\text{MSY}} = 0.8$. Within each subpanel, three box–whisker plots (Tukey 1977) correspond to the three levels of simulated observation error noted at the left of each panel. (Results on simulations with 5% observation error are intermediate to those for 2% and 15% and are omitted to save space.) Therefore, each box–whisker plot represents a unique combination of trajectory, model shape (ϕ), quantity estimated, estimator, level of F_{MSY}, and amount of simulated observation error. For clarity, graphics vary by the quantity estimated. In plots of $B./B_{\text{MSY}}$ and $F./F_{\text{MSY}}$, estimation error is displayed; in plots of ϕ, the estimates themselves are displayed; in plots of MSY, which can vary widely in scale, estimation error is displayed as a percentage of the true value.

Within each box–whisker plot, the solid diamond is the median result from fitting 200 (or for medium F_{MSY}, 400) data sets; the box (open rectangle) marks the quartiles; the whiskers mark the range of most other observations; and open circles mark "outside values", defined as those further from the corresponding quartile than twice the interquartile range, a nonstandard value chosen here to reduce the number of points labeled as outside. Each plot includes a vertical broken line, representing a perfectly accurate estimate. Each plot also contains a number from 0 to 400, indicating the number of successful estimates obtained from that estimator in that specific case. (Those numbers are identical for the four estimated management quantities.) A value of 200 for low or high F_{MSY} or 400 for medium F_{MSY} indicates 100% success in estimation. Estimation failures included three cases: (1) inability to converge on parameter estimates, (2) parameter estimates at the relatively wide constraints set on parameters, or (3) a PRODFIT result specifying the asymptotic model described above.

To increase legibility, some plots do not display the entire range of percent error in MSY estimates; in those plots, a few far-outside values were repositioned close to the highest value shown (100% error in most cases).

Trajectory 1

Estimates of $B./B_{\text{MSY}}$ from the NM estimator were generally more accurate and precise than those from the EA estimator (Figs. 4*a*–4*c*), sometimes by a considerable margin. The EA estimator generally showed a patterned variation in median error with observation error, showing least accuracy with $F_{\text{MSY}} = 0.1$ (low) and 2% observation error. In contrast, results from NM were quite accurate at all levels of observation error and for all ϕ. Both estimators were usually able to provide estimates, EA slightly more frequently than NM.

Estimates of $F./F_{\text{MSY}}$ showed a slightly different pattern (Figs. 4*d*–4*f*). Precision of the EA estimator was similar to that of the NM estimator for most cases, with the notable exception of high F_{MSY} and low ϕ (lower right subpanel of Fig. 4*f*), in which case EA was extremely imprecise. Accuracy also appears similar between estimators, except for simulated populations with $F_{\text{MSY}} = 0.1$ (low); in those cases, EA estimates were precise but inaccurate.

Estimates of MSY from both estimators were extraordinarily precise, with EA estimates from $\phi = 0.35$ and $F_{\text{MSY}} = 0.8$ (high) being the exception, especially at higher levels of observation error (Figs. 5*a*–5*c*). The NM estimator, and to a lesser degree the EA estimator, exhibited greater precision in estimating MSY as the value of F_{MSY} increased. Accuracy was another question: although NM estimates of MSY were generally accurate, EA tended to overestimate MSY at lower values of F_{MSY}, although the inaccuracy was reduced at higher values of F_{MSY}. On this trajectory, the NM estimator slightly overestimated ϕ when $\phi < 0.5$ and underestimated it when $\phi > 0.5$, a pattern more pronounced at higher levels of observation error. The EA estimates of ϕ were generally more precise than the corresponding NM estimates at low levels of observation error but less precise than NM at higher levels of observation error. Accuracy of EA estimates of ϕ was lower overall and varied more with level of observation error than did NM estimates of ϕ.

Trajectory 2

As for trajectory 1, estimates of $B./B_{\mathrm{MSY}}$ from the NM estimator were generally more accurate, and often more precise, than those from the EA estimator (Figs. 6*a*–6*c*). Indeed, NM estimates were in general well centered on the true values (more accurate), with slight inaccuracy appearing with the highest level of observation error. The precision of the NM estimates appears to increase with increasing values of F_{MSY}. On this trajectory, the EA estimator performed poorly when $F_{\mathrm{MSY}} = 0.1$ (low): EA failed to provide estimates in many cases, and its results were inaccurate and imprecise in other cases. Surprisingly, with this value of F_{MSY}, the ability of EA to provide an estimate in our procedure was best with higher levels of observation error; apparently, its tendency to choose the asymptotic model on data with low F_{MSY} was diminished by greater noise content. The importance of noise in arriving at estimates may explain the poor accuracy and precision in such cases.

Estimates of $F./F_{\mathrm{MSY}}$ from the EA estimator were of poor accuracy on most combinations of F_{MSY}, ϕ, and observation error, with results strongly patterned with those variables (Figs. 6*d*–6*f*). Estimates of $F./F_{\mathrm{MSY}}$ from EA generally were too high, often quite substantially. Estimates from NM generally were well centered on the true values and reasonably precise, with both accuracy and precision apparently varying systematically with ϕ and F_{MSY}.

In estimating MSY on this trajectory, EA performed relatively well when F_{MSY} was medium or high ($F_{\mathrm{MSY}} > 0.1$). Estimates were precise, though slightly less accurate than those from NM (Figs. 7*a*–7*c*), particularly under low levels of observation error. In estimating model shape, however, EA was markedly less successful than NM: EA estimates of ϕ were much less accurate than those from NM and often less precise, although the estimator with better precision varied. Each estimator exhibited some patterning of estimates with level of observation error (mild patterning for NM, more marked patterning for EA), suggesting that observation error contributes to inaccuracy in estimating ϕ. The long left-hand tails of some distributions from EA with $F_{\mathrm{MSY}} = 0.2$ (aggregated into the medium group in the figure) suggest that the estimator tends toward the asymptotic model in those cases.

Trajectory 3

This trajectory proved a more difficult challenge, particularly for the EA estimator. Interestingly, the two estimators most often failed to provide results on different sets of cases: NM tended to fail with $\phi = 0.65$ and the highest levels of observation error (but for all values of F_{MSY}), whereas EA tended to fail with lower ϕ, lower F_{MSY}, and less observation error (Fig. 8). Overall, estimates of $B./B_{\mathrm{MSY}}$ from NM were usually more accurate and precise than those from EA, the exception being at $\phi = 0.65$, higher F_{MSY}, and higher levels of observation error; there, EA performed better (Figs. 8*a*–8*c*).

In estimating $F./F_{\mathrm{MSY}}$, NM also performed better in most cases (Figs. 8*d*–8*f*). The exception, again, was the conjunction of high F_{MSY} and high levels of observation error; in many such cases, NM was more accurate but less precise. However, performance of EA in such cases was not particularly good, either. Both methods estimated $F./F_{\mathrm{MSY}}$ relatively well with $\phi = 0.5$ and $\phi = 0.35$, although EA was less successful at low values of F_{MSY}.

Similar patterns were found in estimating MSY; NM performed better in most cases (Figs. 9*a*–9*c*) and offered more consistent performance. In estimating ϕ, NM tended toward the logistic, especially with higher levels of observation error, but EA tended to be less precise and usually less accurate as well, with results for a particular combination of ϕ and F_{MSY} also depending strongly on the level of observation error (Figs. 9*d*–9*f*).

Trajectory 4

On this trajectory, the NM estimator outperformed EA in estimating $B./B_{\mathrm{MSY}}$ (Figs. 10*a*–10*c*) and $F./F_{\mathrm{MSY}}$ (Figs. 10*d*–10*f*) in every scenario. The EA estimator suffered from many estimation failures, severe inaccuracy, and poor precision. In several cases, EA displayed the counter-intuitive pattern of improved accuracy with increasing level of observation error. In contrast, NM estimates were well centered in all scenarios, and estimation precision was high, systematically decreasing as the level of observation error in the data increased.

As one might expect, those patterns also were found in estimates of MSY. The NM estimator performed well generally, whereas EA performed well only when F_{MSY} was high and $\phi \geq 0.5$ (Figs. 11*a*–11*c*). In estimating model shape, NM results were generally well centered about the true values, whereas EA results showed systematic and severe inaccuracies and were often less precise, in some cases exhibiting slight bimodality (Figs. 11*d*–11*f*).

Trajectory 5

In analyzing trajectory 5, the EA estimator more frequently failed to provide estimates than did NM. Comparative performance in estimating $B./B_{\mathrm{MSY}}$ and $F./F_{\mathrm{MSY}}$ was similar to that on trajectory 4: NM markedly outperformed EA in both accuracy and precision (Fig. 12). The EA estimator offered competitive performance only in a few scenarios with higher values of F_{MSY}.

In estimating MSY on this trajectory, both estimators performed well when $F_{\mathrm{MSY}} \geq 0.4$, with NM appearing slightly more accurate. For lower F_{MSY}, NM performed far better than EA, which (as noted) failed in many cases and exhibited poor accuracy in others (Figs. 13*a*–13*c*). In estimating model shape, neither model was outstanding (Figs. 13*d*–13*f*), but NM performed better in most cases. As in previous trajectories, NM showed some lack of accuracy, tending toward the logistic in cases with $\phi = 0.35$ and higher observation errors. However, in most cases, EA results were worse.

Summary results

Relative performance of the two estimators is summarized in a plot of median percent unsigned error (MPUE) of one estimator against the other (Fig. 14). Each point represents MPUE computed on 200 runs of each estimator, all on the same master trajectory, shape of production curve ϕ, value of F_{MSY}, and amount of simulated observation error. Because some runs did not succeed, the corresponding points repre-

Fig. 4. Results for master trajectory 1. Errors in estimates of final-year relative biomass ($B./B_{MSY}$) and relative fishing mortality rate ($F./F_{MSY}$) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

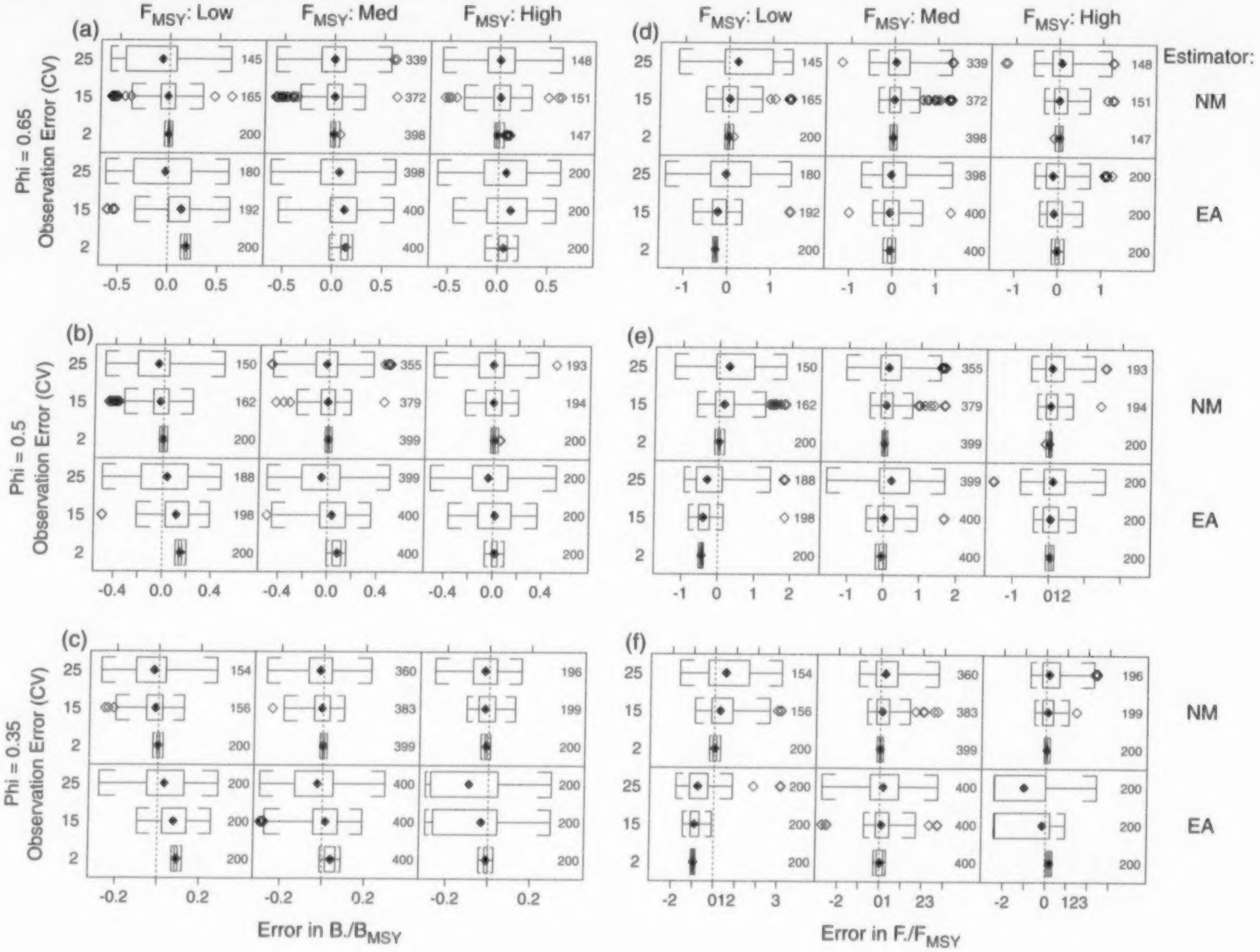


Fig. 5. Results for master trajectory 1. Errors in estimates of maximum sustainable yield (MSY) production-curve shape (ϕ) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

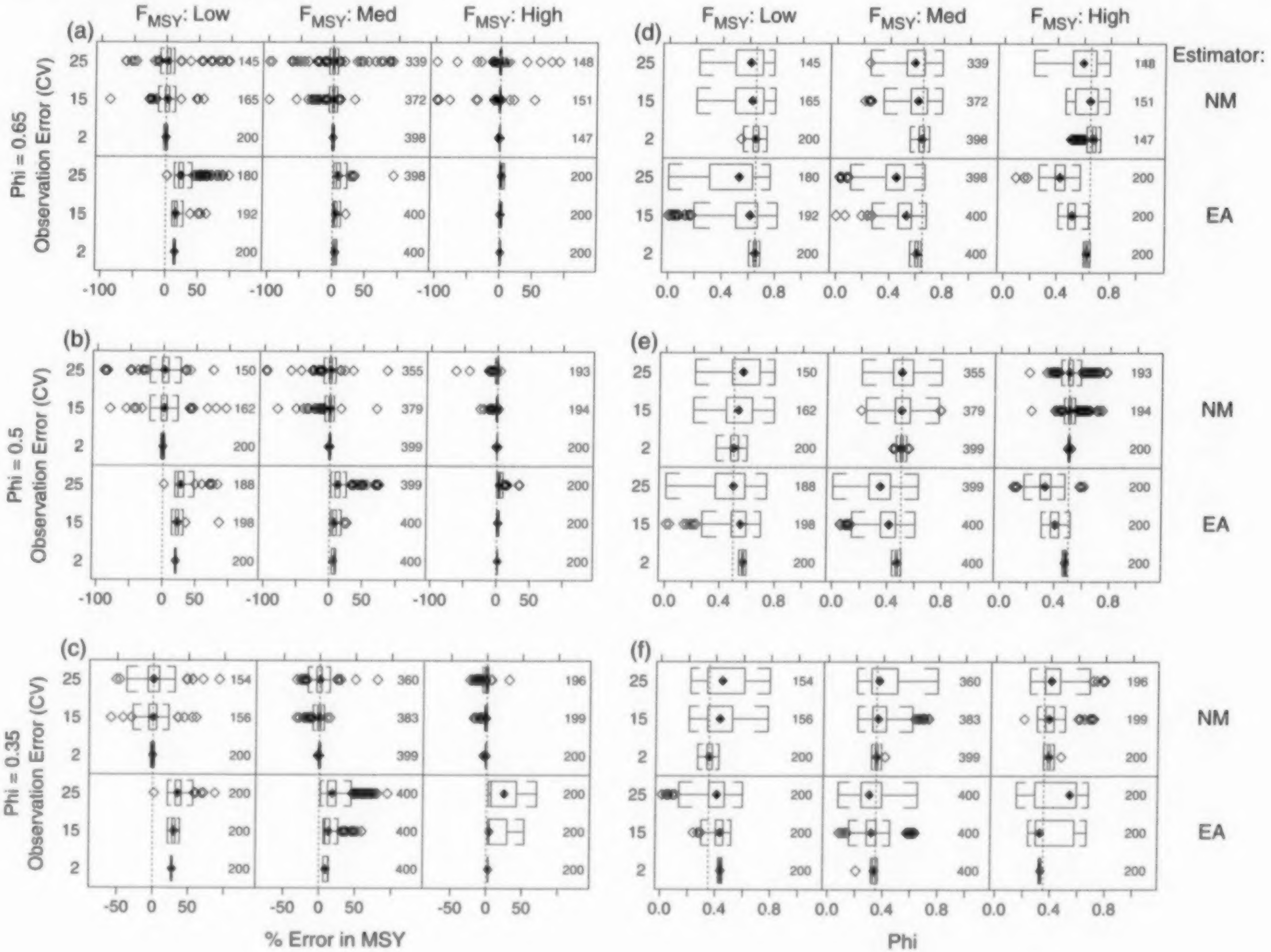


Fig. 6. Results for master trajectory 2. Errors in estimates of final-year relative biomass ($B./B_{MSY}$) and relative fishing mortality rate ($F./F_{MSY}$) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

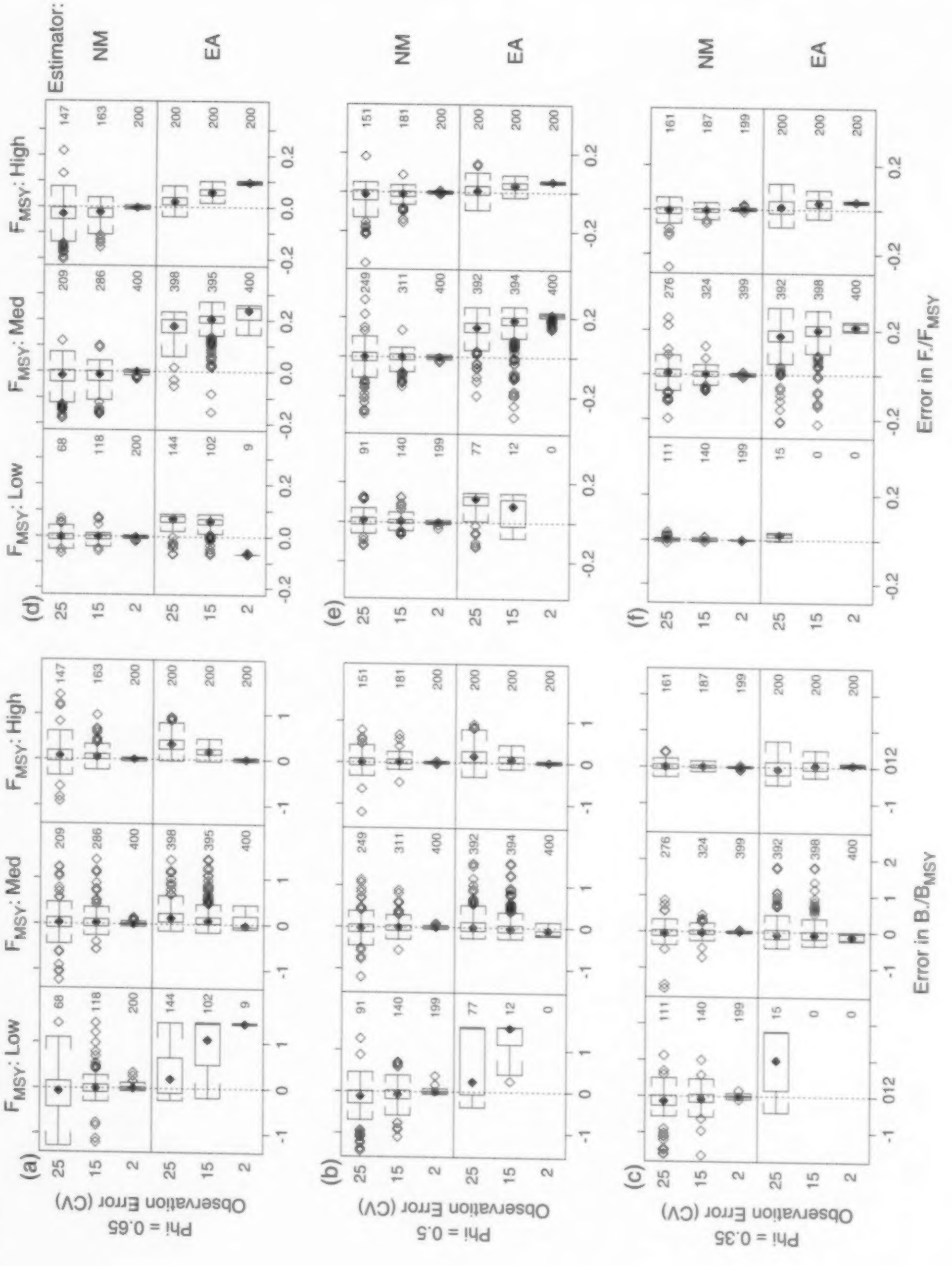


Fig. 7. Results for master trajectory 2. Errors in estimates of maximum sustainable yield (MSY) production-curve shape (ϕ) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

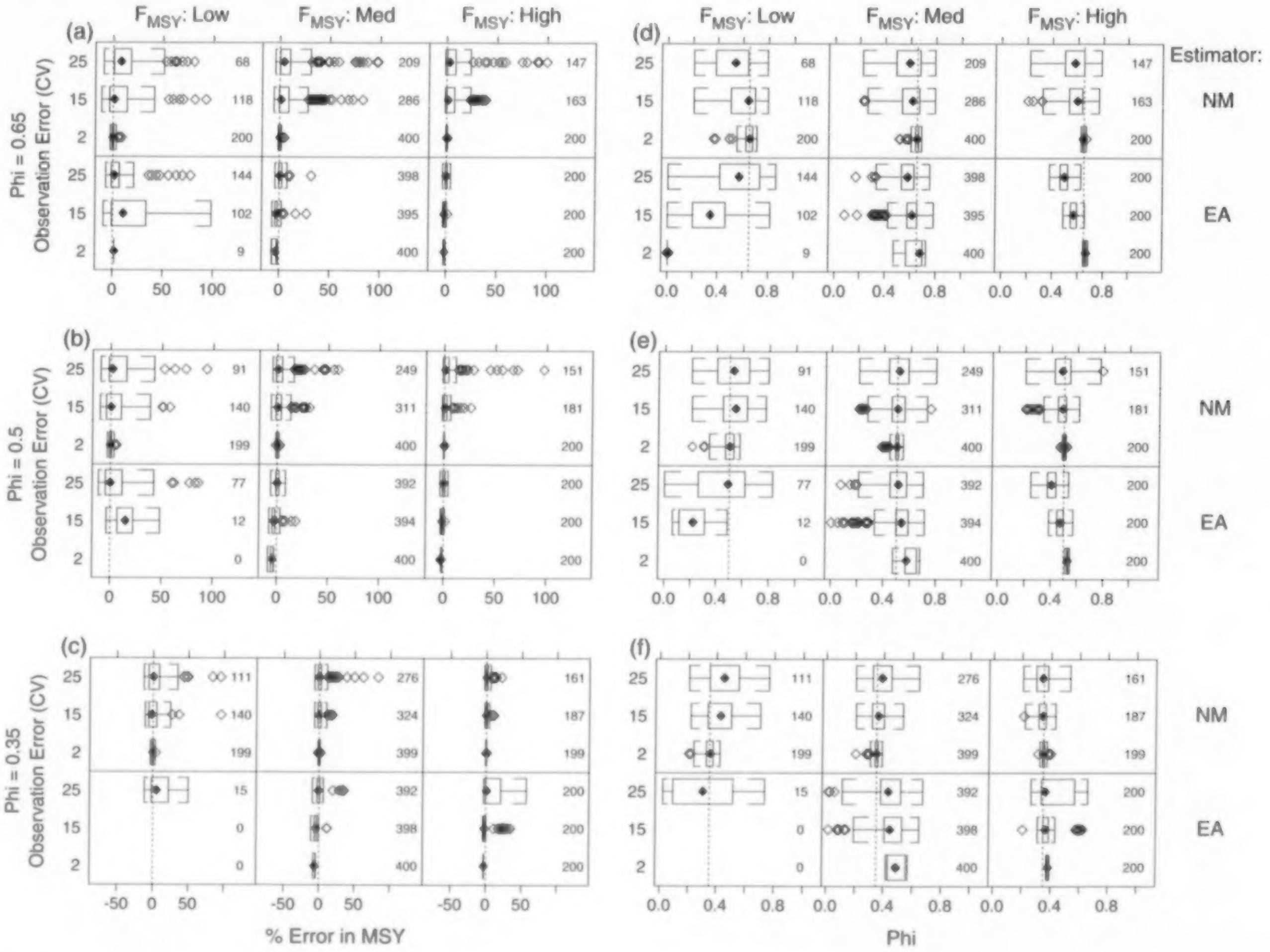


Fig. 8. Results for master trajectory 3. Errors in estimates of final-year relative biomass ($B./B_{MSY}$) and relative fishing mortality rate ($F./F_{MSY}$) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

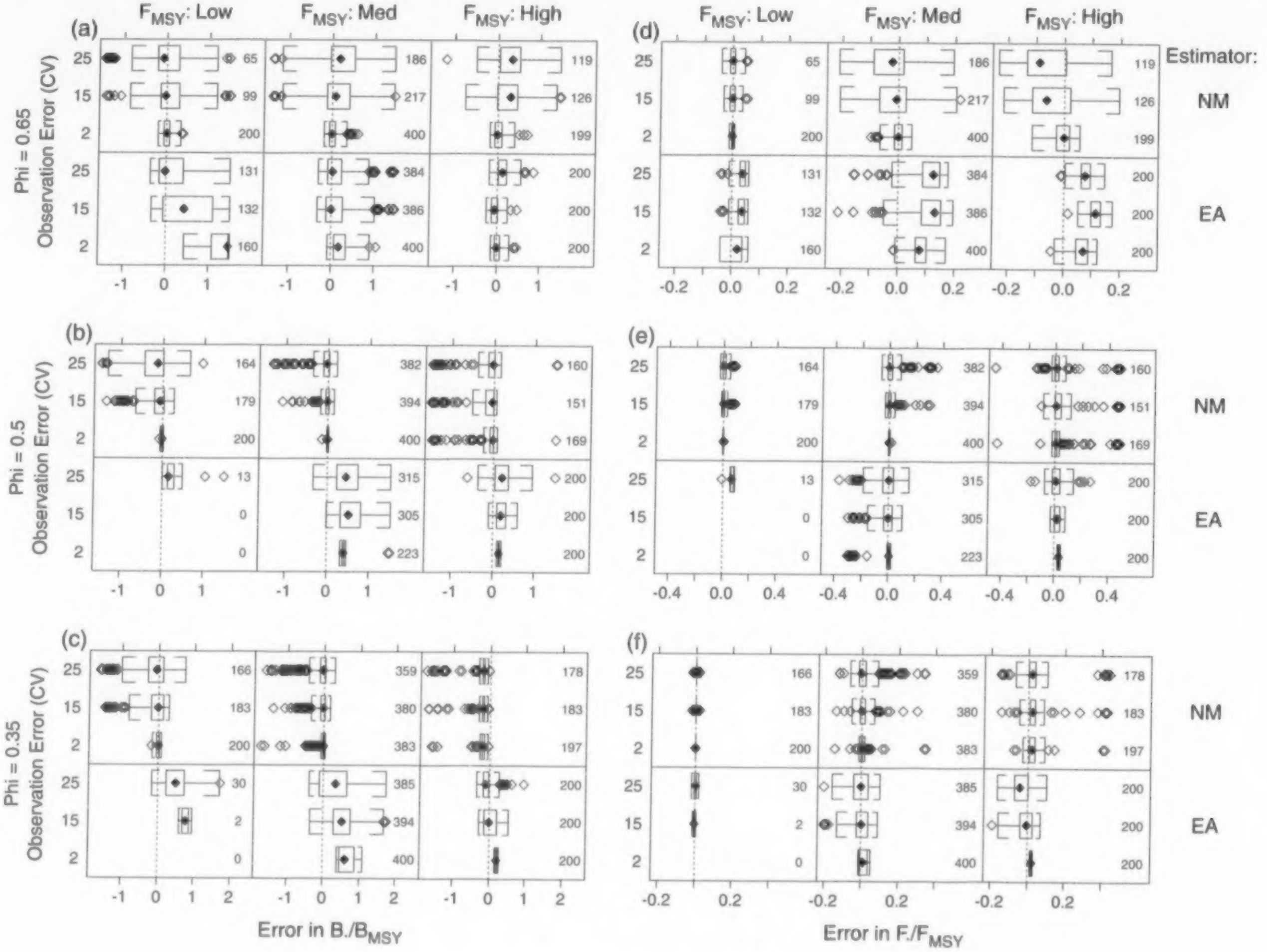


Fig. 9. Results for master trajectory 3. Errors in estimates of maximum sustainable yield (MSY) production-curve shape (ϕ) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

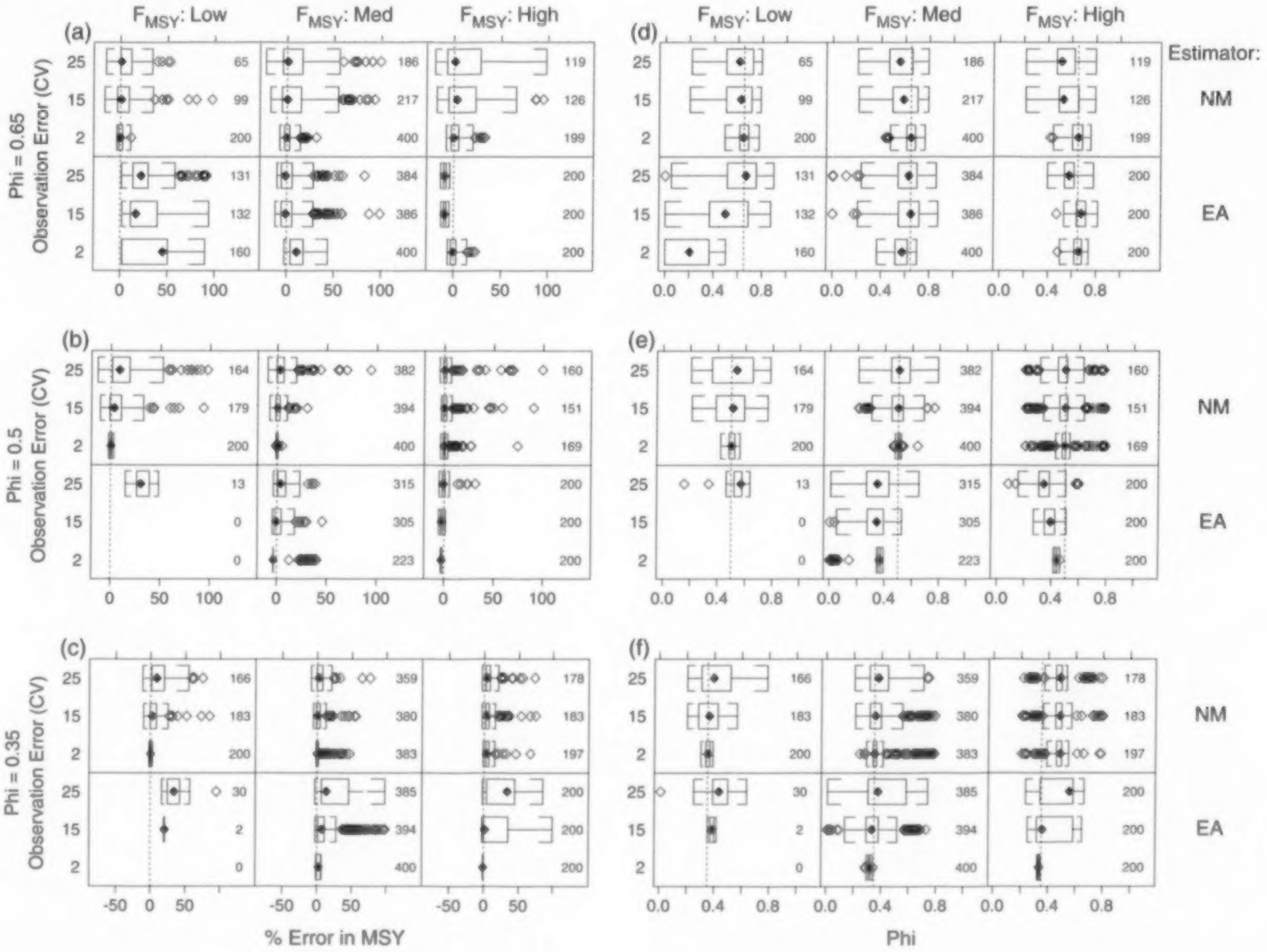
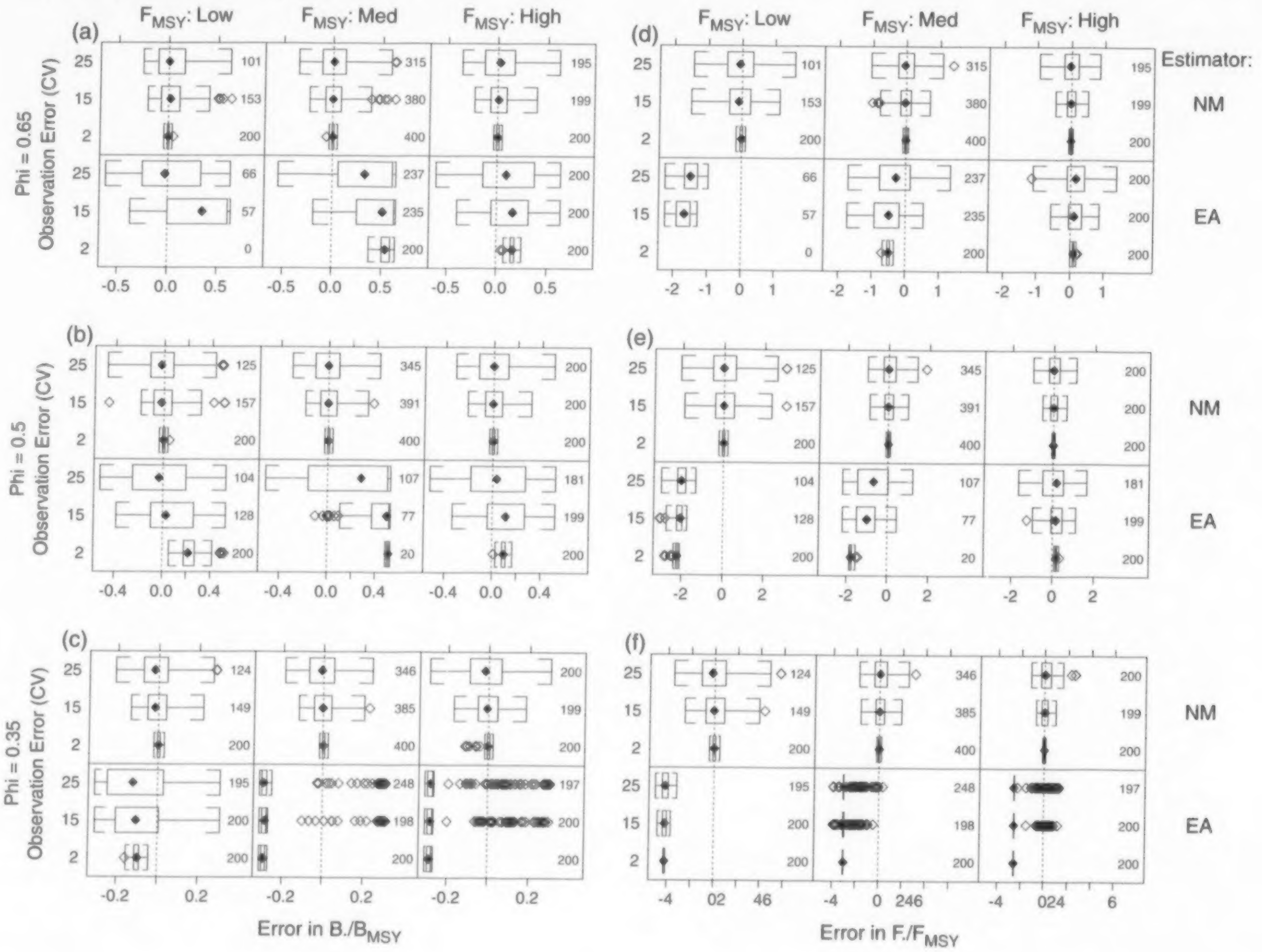


Fig. 10. Results for master trajectory 4. Errors in estimates of final-year relative biomass ($B./B_{MSY}$) and relative fishing mortality rate ($F./F_{MSY}$) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.



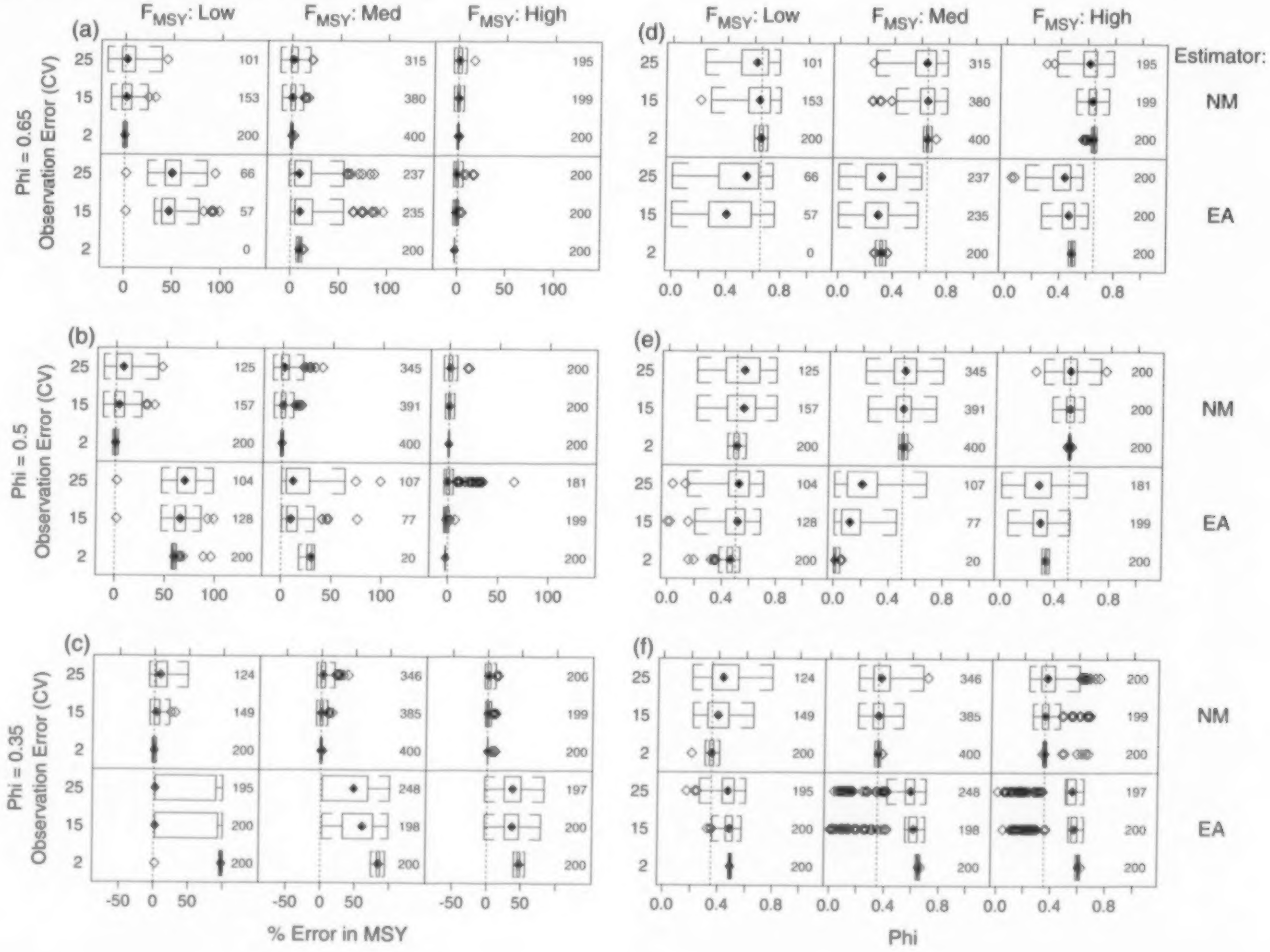


Fig. 11. Results for master trajectory 4. Errors in estimates of maximum sustainable yield (MSY) production-curve shape (ϕ) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

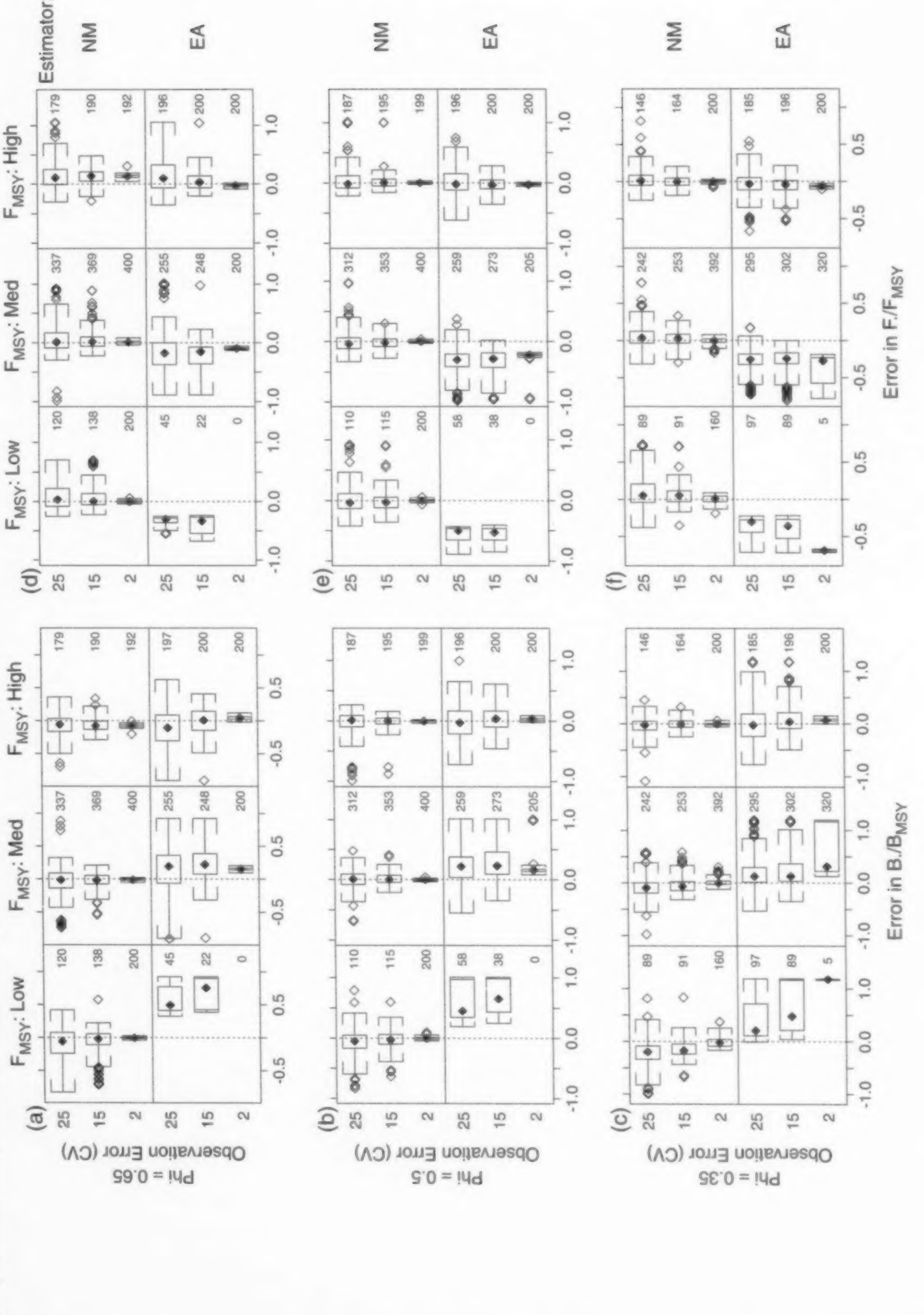


Fig. 12. Results for master trajectory 5. Errors in estimates of final-year relative biomass ($B./B_{\text{MSY}}$) and relative fishing mortality rate ($F./F_{\text{MSY}}$) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

Fig. 13. Results for master trajectory 5. Errors in estimates of maximum sustainable yield (MSY) production-curve shape (ϕ) from two estimators of generalized production model. See text for detailed explanation of plot and simulations.

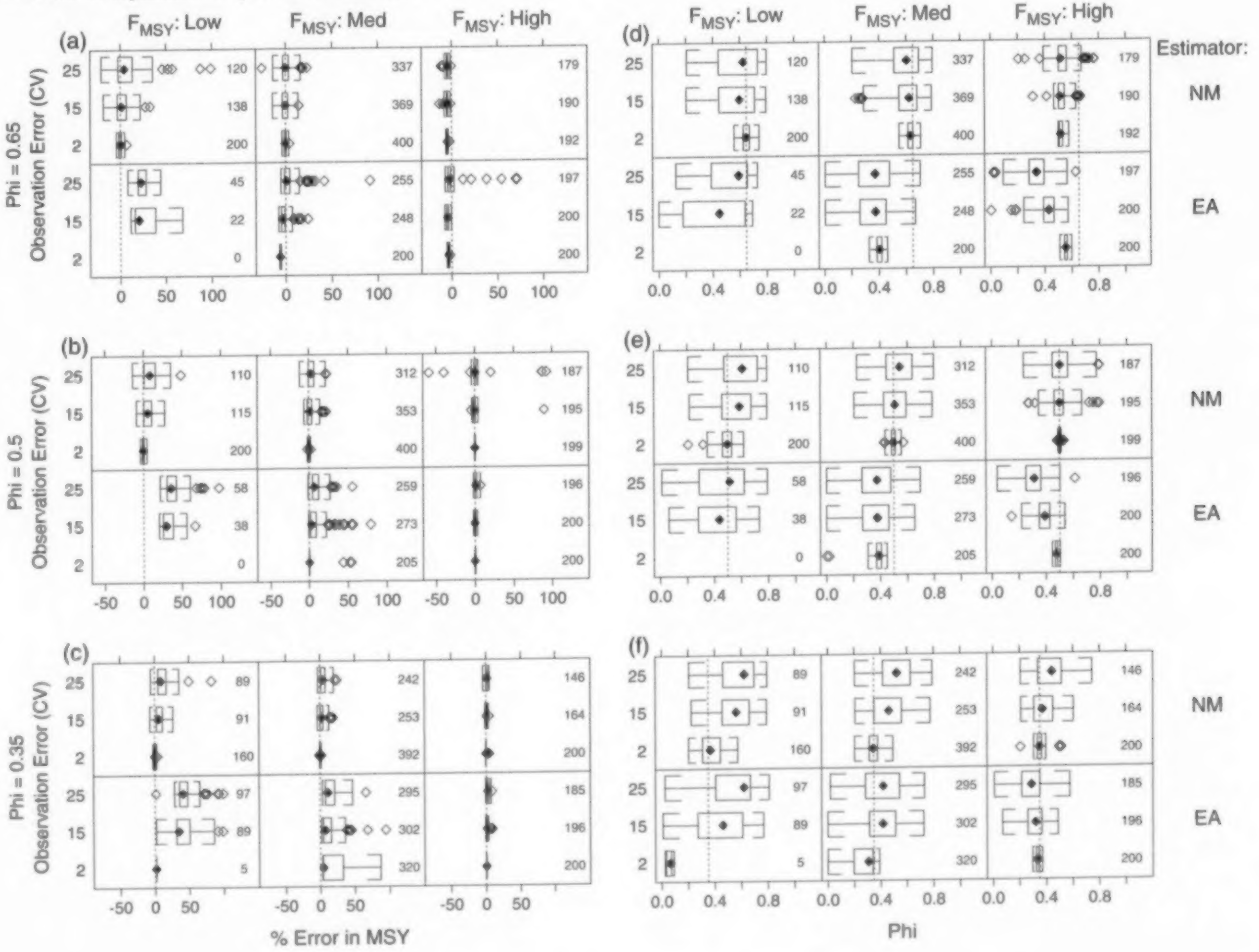
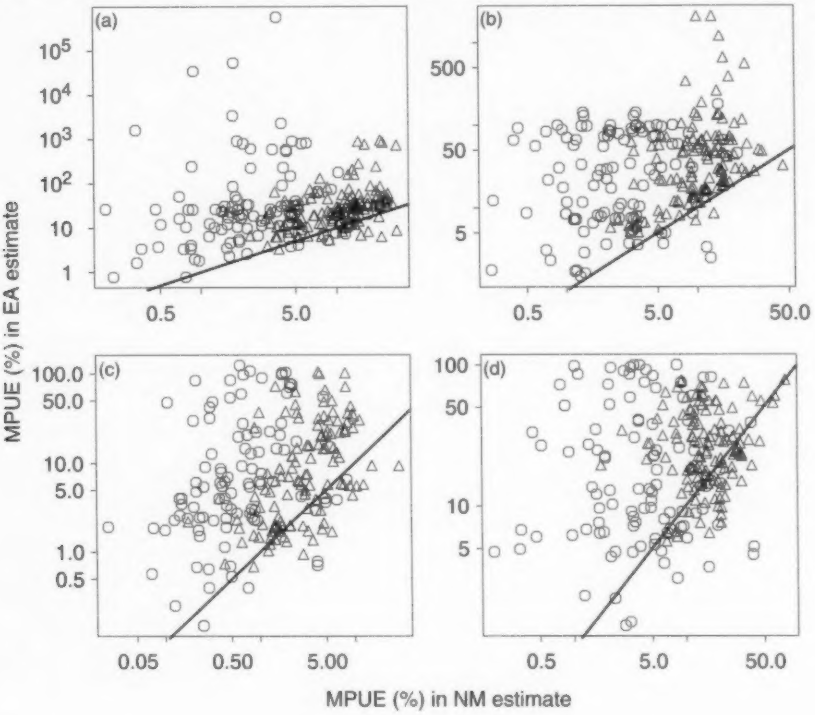


Fig. 14. Summary comparison of estimators for generalized production model. Each point summarizes 200 pairs of estimates (fewer where estimation failures occurred) with median unsigned percent error statistic (MPUE). *Y* coordinate is MPUE from equilibrium estimator (EA); *X* coordinate, from nonequilibrium estimator (NM); line is $Y = X$. Circles, low observation error (2 or 5%) in simulated data; triangles, high observation error (15 or 25%). Quantities estimated (defined in text) are (*a*) $B_./B_{\mathrm{MSY}}$, (*b*) $F_./F_{\mathrm{MSY}}$, (*c*) MSY, (*d*) model shape ϕ. Points above or to the left of the line indicate better performance (lower MPUE) by NM estimator than by EA estimator.



sent fewer than 200 estimates, as per the sample sizes given (Figs. 4–13). We use MPUE as a resistant measure of estimation merit that accounts for both accuracy and precision (Prager 2002). For a set of *N* estimates of quantity *x* with true value = x^*, MPUE is defined as

$$(2)\qquad \mathrm{MPUE}(x) = \mathrm{median}_{n=1}^{N}\left|\frac{\hat{x}_n - x^*}{x^*}\right| \times 100$$

The interpretation of MPUE is that, on similar data, estimation error without respect to sign will be worse than MPUE about half of the time. This examination of results indicates far better performance by the NM estimator overall. In addition to giving lower MPUE than EA in most cases, NM estimates were characterized by low MPUE with low-noise data and MPUE increasing with the noise level in the data (symbol shapes in Fig. 14). In contrast, EA often provided high MPUE even from low-noise data. Superiority of NM was particularly striking in calculation of fishery status ($F_./F_{\mathrm{MSY}}$); in 231 of 240 cases, the NM estimator offered lower MPUE.

Discussion

In any estimation problem, one expects the information content of the data to influence the precision, and possibly the accuracy, of results. Here, the simulated stocks with higher F_{MSY} are by definition more dynamic than those with lower F_{MSY}. (Under the logistic production model, the definition $r = 2F_{\mathrm{MSY}}$, where *r* is the population's intrinsic rate of increase, illustrates this clearly.) It follows that simulated data sets representing the former should provide more information on response to exploitation than simulated data on the latter and thus should support better estimates. That view seems to be confirmed by the systematic increase of precision from series of equivalent simulated data with increasing values of F_{MSY}, i.e., the increased precision as one moves to the right in most rows of Figs. 4*a*–13*f*.

The overall pattern of each master trajectory provides the core information used in parameter estimation. In this study, trajectories 3 and 4 provided estimates with the worst overall accuracy and precision. That seemed odd to us at first, as each of those trajectories covers a wide range of population size and each crosses $B = B_{\mathrm{MSY}}$ twice; they would seem to be information-rich. However, trajectories 1 and 2, which provided better accuracy and precision, also included sustained time periods (roughly years 13–25) of biomass levels near B_{MSY}. We conclude that not only contrast in the data, but also sufficient sampling intensity at each region of population size, is important in parameter estimation for production models.

A referee viewed our study as a case history in nonlinear regression based experimental design. From that perspective, only certain designs (patterns of exploitation rates and abundances over time) can produce accurate and precise parameter estimates for our nonlinear model, and description of such designs may not have much in common with classification of trajectories as increasing, decreasing, and so on. That is an interesting thought and one explanation for the complexity of our results.

An unexpected pattern occasionally observed in EA estimates was the tendency toward improved accuracy with increasing observation error (e.g., Fig. 4*a*, lower left subpanel). We note that the degree and direction of inaccuracy in EA estimates often seemed to depend systematically on the combination of F_{MSY} and the observation-error level, but beyond that we can say little to explain the observed pattern.

The remark of Sissenwine (1978) that equilibrium estimators can readily produce answers, albeit ill-founded ones, was directed to estimation practices in the 1970s for the logistic production model. If instead the Fox (1975*a*) equilibrium estimator for the generalized production model is used, and if one rejects the asymptotic model (as here), success in estimation is no longer guaranteed. However, our results agree with Sissenwine's impression that the quality of estimates from equilibrium methods can be poor.

Equilibrium methods repeatedly have been found to be biased; the fisheries literature includes recommendations against them. We examined the question again in the first study to compare estimators for the generalized production model, and with the most comprehensive set of simulations to date. Our simulated data sets covered wide ranges of stock productivity, shape of the production curve, and observation error, while also considering a broad range of possible time trajectories of stock biomass. We found it impossible to discern a priori from a data set whether the equilibrium estimator would perform acceptably, and in general, the equilibrium estimator performed poorly in estimating parameters of the generalized production model and, as a special case, parameters of the logistic production model. In contrast, a more modern observation-error estimator, although not perfect, showed better accuracy and precision. Equilibrium methods played an important role when computer power was far less available, but there is no reason to use them today. We conclude that equilibrium methods should be abandoned and abandoned completely. We hope that the scope of our simulations provides sufficient evidence for even former skeptics to adopt that recommendation.

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The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr

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Abstract: This study combines laboratory experiments with temperature monitoring and fish sampling in the wild to determine if Atlantic salmon (*Salmo salar*) parr from the Miramichi River in New Brunswick are currently experiencing significant sublethal heat stress during the warm summer months. Laboratory experiments indicated that Hsp 70 mRNA and protein and Hsp 30 mRNA were all significantly induced in Atlantic salmon parr between 22°C and 25°C. Field sampling during moderate spring temperatures and a high-temperature event in summer further indicated that the threshold for mRNA induction of both Hsp 70 and Hsp 30 is around 23°C, but Hsp 70 protein levels were only significantly elevated in the field at 27°C. Hsc 70 mRNA and protein levels were not significantly increased during heat stress under laboratory conditions. In the field, however, Hsc 70 mRNA was significantly increased at 23°C and both Hsc 70 mRNA and protein levels were elevated at 27°C. Taken together, the results of this investigation suggest that Atlantic salmon parr from the Miramichi River are currently experiencing temperatures that will cause significant protein damage and induce a heat-shock response for about 30 days each summer.

Résumé : Notre étude combine des expériences en laboratoire, des enregistrements de température et des échantillonnages de poissons en nature dans le but de déterminer si les tacons du saumon de l'Atlantique (*Salmo salar*) de la Miramichi au Nouveau-Brunswick subissent actuellement un stress thermique sublétal significatif durant les mois les plus chauds. Les expériences de laboratoire indiquent que des quantités significatives d'ARNm Hsp 70, de protéine Hsp 70 et d'ARNm Hsp 30 sont produites chez les tacons du saumon de l'Atlantique entre 22 et 25°C. Des échantillonnages sur le terrain durant des périodes de température modérée au printemps et durant une période de chaleur en été montrent, de plus, que le seuil pour la production des ARNm Hsp 70 et 30 se situe à environ 23°C; toutefois, les concentrations de protéines Hsp 70 ne s'accroissent significativement en nature qu'à 27°C. Les concentrations d'ARNm Hsc 70 et de protéine Hsc 70 n'augmentent pas significativement durant un stress thermique en laboratoire. En nature, cependant, les concentrations d'ARNm Hsc 70 sont significativement plus élevées à 23°C et celles de l'ARNm et de la protéine Hsc 70 le sont à 27°C. Dans leur ensemble, nos résultats laissent croire que les tacons du saumon de l'Atlantique de la Miramichi subissent actuellement des températures qui causent des dommages significatifs aux protéines et provoquent des réactions de choc thermique durant une trentaine de jours chaque été.

[Traduit par la Rédaction]

Introduction

The Atlantic salmon (*Salmo salar*) is one of Canada's most important recreational fish. In recent years, population declines have resulted in reductions in Atlantic salmon sport fisheries and a complete closure of the commercial fishery in eastern Canada. Atlantic salmon are now classified as an endangered species in parts of Canada (Bay of Fundy) and the United States (northern Maine). Reasons for the observed population declines are believed to be many and include variables such as overfishing, pollution, habitat degradation, and temperature stress.

Water temperature is known to be one of the most important environmental variables affecting fish. Although temperature stress is often associated with human activities such as deforestation and thermal pollution, it can also occur naturally as a result of changes in natural stream characteristics or meteorological conditions. High stream temperatures between 23°C and 25°C have been observed to cause mortality in trout populations (Lee and Rinne 1980; Bjornn and Reiser 1991).

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Although Atlantic salmon can tolerate slightly higher temperatures, 27–28°C, depending on their life stage (Huntsman 1942; Garside 1973), the sublethal impacts of these temperatures on molecular and physiological processes within the various life-history stages of Atlantic salmon are less well understood.

Previous studies have shown that behavioural changes in Atlantic salmon start to occur at temperatures well below their lethal maximum, in the range of 22–24°C, when they start searching for refuge (Cunjak et al. 1993). Although these behavioural changes are helpful in determining stressful temperatures for fish, changes on a molecular level may be the first "early warning signals" of sublethal heat stress that will later be manifested at an organismal or population level. One of the most common molecular indicators, or biomarkers, of temperature stress in all organisms is the heat-shock response. This response is characterized by a dramatic change in the pattern of gene expression resulting in a rapid induction of heat-shock mRNA and protein translation and simultaneous repression of synthesis of other cellular proteins (Lindquist 1986). The number and type of heat-shock proteins (hsps) induced in response to temperature stress is both species and tissue specific. Members of the Hsp 70 family are highly temperature responsive and are the most conserved of all hsps known (Sanders 1993). Constitutive or cognate members such as Hsc 70 act during non-stressful conditions to prevent protein aggregation and to facilitate proper protein folding and translocation between cellular compartments. In addition to all of their constitutive roles, stress-inducible members such as Hsp 70 act to solubilize or renature proteins that have been denatured, translocate irreversibly damaged proteins to lysosomes and proteasomes for degradation, and confer protection to vital cellular functions. Small hsps such as Hsp 30 are also induced in response to thermal stress and are thought to be present constitutively in unstressed cells (Arrigo and Landry 1994). Small hsps have also been shown to help protect actin microfilaments in the cell following stress (Lavoie et al. 1993; Benndorf et al. 1994; Huot et al. 1996).

Because transcription of relevant hsp mRNAs may be induced 50- to 100-fold within minutes of a temperature increase (Lis and Wu 1994), their measurement may provide rapid and sensitive information about the degree of thermal stress that a fish may be experiencing in the wild. Although the subsequent translation of relevant hsp proteins may be slightly delayed by comparison, their value as indicators of sublethal heat stress may lie in their longevity in comparison to their respective mRNAs. Hsp protein levels may therefore be more valuable as indicators of long-term temperature stress. Although the half-life of hsps is dependent on the tissue and organism, previous studies have shown that Hsp 70 has a half-life of at least 4 days in most organisms (Lindquist 1986; Sanders 1990). In addition to behavioural responses, molecular indicators such as hsps can therefore provide valuable insights about disturbances that are causing significant sublethal stress in fish.

In recent years, climate change has been identified as an important source of stress in aquatic ecosystems (Sinokrot et al. 1995). In parts of North America, many fish at the northern extremes of their distributional range are now experiencing temperatures close to their upper lethal limits (Eaton et al. 1995; Sinokrot et al. 1995). Global warming models for eastern Canada also predict that climate change may result in an overall loss of juvenile Atlantic salmon habitat (Minns et al. 1995).

Despite the fact that many North American fish populations may currently be affected by warm temperatures in their aquatic ecosystems, few studies have examined both the thermal behaviour of these systems and the biological impacts of warm temperatures on resident species. The goal of the present study was therefore to further examine this issue on one of Canada's most important Atlantic salmon river systems, the Miramichi. More specifically, we examine the thermal characteristics of two Miramichi rivers, one large and one small, during summer heat stress conditions and determine whether or not summer temperatures are causing enough sublethal thermal stress to induce a heat-shock response in Atlantic salmon parr. Natural heat-shock responses for Miramichi Atlantic salmon in the field are also compared with heat shock induction profiles obtained under controlled laboratory conditions.

Materials and methods

Series I. Experimental test of heat shock induction temperature

Animals

Twenty-six Atlantic salmon parr (*Salmo salar*) were obtained by electrofishing from Stewart Brook on 3 and 4 August 2000. Stewart Brook is a tributary of the Miramichi River and is located adjacent to the Miramichi Salmon Hatchery in South Esk, New Brunswick. Ten of these fish were transferred to a 7.5°C holding tank, and sixteen were placed in a holding tank at the ambient brook temperature of 16°C. The 7.5°C holding tank was supplied with aerated well water, whereas the 16°C tank was supplied with aerated brook water. Both holding tanks were situated inside the Miramichi Hatchery on a schedule of 12 h light – 12 h dark. Salmon were not fed during the course of the experiment. It is noteworthy that a control experiment conducted in a parallel study with brook trout (*Salvelinus fontinalis*) showed that electrofishing does not cause induction of Hsp 70 or Hsp 30 mRNA.

Experimental design

Salmon parr in the 16°C holding tank were maintained at a constant temperature for 30 h before experimentation. At the beginning of the experiment, four fish were sampled at the ambient temperature of 16°C. The water in the tank was then warmed to 22°C over a 1-h period and then held at that temperature for 2 h. Four salmon were then transferred to an adjacent recovery tank at the ambient brook temperature of 16°C, where they remained for 1 h before sampling. This procedure was repeated for the temperatures of 25°C and 28°C. The 10 fish placed at 7.5°C were maintained at that temperature for 5 days and then sampled as cold-water controls. During sampling, fish were removed quickly from the tank and were terminally anaesthetized in water containing 250 $mg \cdot L^{-1}$ 3-aminobenzoic acid ethyl ester (MS-222, Sigma-Aldrich Corp., St. Louis, Mo.) buffered with 500 $mg \cdot L^{-1}$ $NaHCO_3$.

Salmon lengths were recorded, followed by decapitation and removal of internal organs. The remaining torsos (white and red muscle, bone, and skin) were then wrapped in aluminum foil and immediately frozen in liquid nitrogen until they were processed for RNA and protein analysis.

Series II. Field heat stress studies

Animals and experimental design

Atlantic salmon parr were sampled from three sites in the Miramichi River system, once during the late spring and once during a natural high-temperature event later in the summer. For the late spring condition, seven parr were collected by electrofishing from each of Otter Brook (OB; 17.5°C), Little Southwest Miramichi River above the confluence of Catamaran Brook (LSW; 20°C), and the lower reach of Catamaran Brook (CAT; 22°C) on 19 June 2000. During the high-temperature event, parr were collected, also by electrofishing, from Little Southwest Miramichi River above the confluence of Catamaran Brook (27°C) and from the middle reach of Catamaran Brook (23°C) on 3 August 2001. Temperatures noted here represent the actual temperature in the river recorded with a precision field thermometer (±0.5°C) at the time of sampling. Parr from Otter Brook were not sampled during the high-temperature event because water temperature was only 0.5°C higher than during the late spring condition. Samples were taken from the middle reach of Catamaran Brook during the high-temperature event as opposed to the lower reach because water levels were low and other experiments were being conducted within the lower reach on the sampling day. Fish from each location were held in flow-through boxes within the river or brook until all seven parr were collected. All fish from each site were then terminally anaesthetized in brook or river water containing 250 $\text{mg}\cdot\text{L}^{-1}$ MS-222, buffered with 500 $\text{mg}\cdot\text{L}^{-1}$ $NaHCO_3$. Salmon were then decapitated and their internal organs were removed. The remaining torsos (white and red muscle, bone, and skin) were then wrapped in aluminum foil and immediately frozen on dry ice (late spring controls) or in liquid nitrogen (high-temperature event) until they were processed for RNA and protein analysis.

Series III. Field temperature measurements

In the present study, water temperature data were obtained from both the Catamaran Brook (middle reach and lower reach) and Little Southwest Miramichi River. The selection of these sites reflects the range of river thermal characteristics observed within the Miramichi River basin. Catamaran Brook is a relatively small stream with a drainage basin of 52 km^2. The lower reach represents the lower 5.5 km of stream, the middle reach, km 8.3 to km 15.5. Catamaran Brook is typically 10–15 m wide with relatively good streamside canopy. In contrast, Little Southwest Miramichi River (at the confluence of Catamaran Brook) represents larger river systems within the Miramichi catchment and has a basin drainage area of 1190 km^2. Little Southwest Miramichi River, having a width of approximately 80 m, is typical of rivers in the region and is characteristically wide and shallow, with coarse substrate.

Hourly temperatures were recorded at both sites using a CR10 Campbell Scientific datalogger (Campbell Scientific, Inc., Logan, Utah). Datalogger sensors were installed in well-mixed zones within the rivers, i.e., where lateral (cross-sectional) temperatures are uniform. To quantify the potential heat stress resulting from high temperatures, the number of days that the water temperature exceeded 23°C was counted. For comparison, the number of days in which air temperatures exceeded 25°C was also compiled using long-term data collected at the Catamaran Brook meteorological station. This meteorological station is situated in the centre of the basin and thus provides a good indication of thermal condition for the whole basin.

RNA isolation and northern blot analysis

Total RNA from series I and II was extracted by the acid–phenol method as previously described (Currie at al. 2000). Two washes in 4 $\text{mol}\cdot\text{L}^{-1}$ LiCl (diethylpyrocarbonate-treated) were incorporated into the method, before the final resuspension of the RNA, to help reduce glycogen contamination. Total RNA was quantified in duplicate using a Spectramax plate spectrophotometer (Molecular Devices Corp., Sunnyvale, Calif.). For northern blots, 10 µg of total RNA was fractionated by glyoxal–dimethyl sulphoxide (DMSO) denaturing electrophoresis on a 1% agarose gel and transferred to a nylon membrane (Stratagene Duralon; Stratagene, La Jolla, Calif.) using a 20× standard saline citrate (SSC). Membranes were ultraviolet crosslinked (UV crosslinker; Fisher Scientific, Pittsburgh, Pa.) twice at optimal setting before hybridization.

Probes for Hsp 70 mRNA, heat-shock cognate 71 (Hsc 71) mRNA, and Hsp 30 mRNA were as described previously (Currie at al. 1999, 2000). For purposes of clarity, and to prevent confusion between the Hsc 70 antibody and mRNA probe, Hsc 71 mRNA will herein be referred to as Hsc 70 mRNA. Drosophila α-tubulin, used as a non-stress indicator of mRNA synthesis, was generously provided by Dr. C. Moyes (Queen's University, Kingston, Ont.). All probes were labelled using [α-^{32}P]dCTP (specific activity 10^9 $\text{counts}\cdot\text{min}^{-1}\cdot\mu\text{g}^{-1}$ DNA) and the Ready-To-Go labelling system (Pharmacia Corp., Peapack, N.J.). Membranes were prehybridized at 68°C for 30 min with QuickHyb hybridization solution (Stratagene). Blots were hybridized for 2 h in the same solution at 68°C, with approximately 10^9 $\text{counts}\cdot\text{min}^{-1}$ of denatured probe. Blots were then washed twice with a 1× SSC – 0.1% SDS solution (15 min, 26°C) followed by an additional wash in 0.25× SSC – 0.1% SDS (30 min, 68°C). Blots were exposed to a phosphor screen (Eastman Kodak Co., Rochester, N.Y.) and then visualized and quantified using a phosphoimager driven by ImageQuant software (Molecular Dyanmics, Sunnyvale, Calif.). Band densities were expressed as a ratio of the band with the greatest intensity on each separate blot. All membranes were probed with a human 18S rRNA to correct blots for loading differences. Quantitative comparisons between laboratory and field samples were made possible by running the heat-shock (28°C) laboratory samples a second time through northern hybridization concurrent with the field samples.

Protein sample preparation, SDS–PAGE, and western blotting

Protein samples were prepared by sonicating approximately 30 mg of powdered whole body tissue in 200 µL of ice-cold Tris buffer (50 mM, pH 7.5) treated with a protease inhibitor cocktail containing 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF), sodium EDTA, bestatin, *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane (E-64), leupeptin, and aprotinin (Sigma-Aldrich Corp., St. Louis, Mo.). Homogenates were centrifuged (14 000 rpm for 1 min), and 150 μL of supernatant was added to an equal volume of 2× Laemmli's buffer (0.35 M SDS, 0.58 M sucrose, 0.289 mM bromophenol blue, 125 mM Tris, and 0.05% β-mercaptoethanol). Total protein for each sample was assayed in duplicate using the bicinchoninic acid (BCA) protein assay reagent method (Pierce Chemical Co., Rockford, Ill.).

Protein samples were diluted to a final concentration of 1.5 $mg \cdot mL^{-1}$ with 1× Laemmli's buffer, boiled, and then loaded onto a one-dimensional SDS polyacrylamide gel (8%) and subjected to electrophoresis (40 μg $protein \cdot lane^{-1}$). To allow for inter-gel comparisons, a standard (28°C heat-shock sample) was loaded in the last lane of every gel. Gels were then transferred (25 V for 30 min) onto nitrocellulose membranes with a Semi-Dry transfer unit (Bio-Rad Laboratories, Inc., Hercules, Calif.) using a transfer buffer (25 mM Tris, 192 mM glycine, and 20% methanol). Membranes were blocked for 1 h with a blocking solution consisting of 5% skim milk and 0.05% sodium azide in TTBS (20 mM Tris (pH 7.5), 300 mM NaCl, 0.01% Tween 20). Membranes were then probed with primary antibody (either Hsp 70 or Hsc 70) in a 1:3000 ratio with blocking solution. The Hsp 70 probe was a polyclonal rainbow trout antibody that recognizes both the inducible and constitutive forms of the 70 kDa stress protein. This antibody was a generous gift from Dr. P. Candido (Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B.C.). The Hsc 70 probe was a polyclonal trout antibody specific to Hsc 70 and did not recognize the inducible form (Boone and Vijayan 2002). The secondary antibody used was alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma-Aldrich), which was also diluted in a ratio of 1:3000 in blocking solution. Blots were incubated with the primary antibody for 1 h at room temperature followed by two washes (1 × 10 min, 1 × 5 min) in TTBS, then one 10-min wash in TBS (TTBS minus Tween 20). Secondary antibody was then added and incubated at room temperature for an additional hour followed by the same washing protocol as used for the primary antibody. Bands were visualized using a detection solution consisting of 49.5 μL Nitro Blue Tetrazolium (NBT; 50 $mg \cdot mL^{-1}$ in 70% dimethylformamide) and 25 μL 5-bromo-4-chloro-3-indolyl-phosphate (BCIP; 50 $mg \cdot mL^{-1}$ in 100% dimethylformamide) in 6.75 mL alkaline phosphatase buffer (100 mM Tris, 100 mM NaCl, and 50 mM $MgCl_2$). The band size was confirmed with prestained low-range molecular markers (Bio-Rad). Blots were scanned and the images quantified using the AlphaEase software package (Alpha Innotech Corp., San Leandro, Calif.). Band densities were expressed as a ratio of the band with the greatest intensity on each separate blot.

Statistical analysis

Heat-shock mRNA and protein data are presented as means (± 1 standard error of the mean) where appropriate. A one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls test for the pairwise comparison of means was used to assess significant differences ($P < 0.05$) in hsp band density.

Results

Heat shock induction temperature

Under experimental conditions, both Hsp 70 mRNA and protein content in salmon parr increased with increasing temperature (Fig. 1). Levels of both Hsp 70 mRNA and protein became significantly greater than controls (7.5°C and 16°C) by 25°C. At 28°C, these variables were about six and eight times greater, respectively, than those measured in the 16°C controls (Fig. 1*a*). Hsp 30 mRNA induction with increasing temperature followed the same pattern as that observed for Hsp 70 (Fig. 2). Hsp 30 mRNA content was also significantly greater than controls (7.5°C and 16°C) by 25°C, and at 28°C, it was over five times greater than that for the 16°C controls (Fig. 2*a*). There was no significant effect of increasing temperature on Hsc 70 mRNA or protein levels in salmon parr that were heat-shocked under laboratory conditions (Fig. 3).

Field heat stress studies

Hsp 70 mRNA content in Atlantic salmon parr sampled directly from both Catamaran Brook and the Little Southwest (LSW) Miramichi River during a high-temperature event (23°C and 27°C, respectively) was significantly higher than that in the late spring control fish from the same sites (Fig. 4). The Hsp 70 mRNA levels in salmon sampled during the high-temperature event were about 2–3 times higher than those in the late spring controls from Catamaran Brook and the LSW Miramichi River, respectively. It is also noteworthy that the Hsp 70 mRNA response during the high-temperature event in the LSW Miramichi River was still less than that observed during exposure to a heat stress of 28°C under laboratory conditions. In contrast to Hsp 70 mRNA, Hsp 70 protein content was not significantly elevated in salmon from Catamaran Brook during the warm-water event. On the LSW, however, there was a significant increase in Hsp 70 protein levels during warm-water conditions (Fig. 4). Notably, the Hsp 70 protein content in Atlantic salmon parr from the LSW during the high-temperature event was also significantly greater than that in salmon parr exposed to a 28°C heat stress in the laboratory experiments.

Atlantic salmon parr from Catamaran Brook and the LSW Miramichi River also exhibited an increase in their Hsp 30 mRNA content during the high-temperature event. The levels of Hsp 30 mRNA in salmon sampled during the high-temperature event were about double those in the late spring control fish (Fig. 5). In contrast to the trend observed for Hsp 70 mRNA, however, the salmon in the LSW Miramichi River contained an equal amount of Hsp 30 mRNA as compared to the salmon sampled at 28°C under experimental conditions in the Miramichi Hatchery.

Hsc 70 mRNA content was significantly higher in Atlantic salmon sampled from both the Catamaran Brook and the LSW Miramichi River during the high-temperature event than during cooler-water conditions of late spring (1.5- and 1.6-fold higher, respectively; Fig. 6). However, the Hsc 70 mRNA contents in salmon sampled during the heat stress conditions in both of these rivers were not significantly different than those observed for Atlantic salmon parr heat-shocked under control conditions at 28°C in the Miramichi Hatchery. To ensure that the trends observed for the field

Fig. 1. (*a*) Relative mRNA (open bars) and protein concentrations (solid bars) of heat-shock protein 70 (Hsp 70) in whole body torsos ($N = 10$ for 7.5°C controls; $N = 4$ for all other temperatures (mRNA); and $N = 4$ for all protein samples) from Atlantic salmon (*Salmo salar*) parr exposed to an acute temperature stress under experimental conditions. Differing letters denote a significant difference within mRNA and protein samples, respectively ($P \le 0.05$). All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern bands for Hsp 70 mRNA induced in Atlantic salmon torsos as described in Fig. 1*a*. (*c*) Representative western bands for Hsp 70 induced in Atlantic salmon torsos as described in Fig. 1*a*.

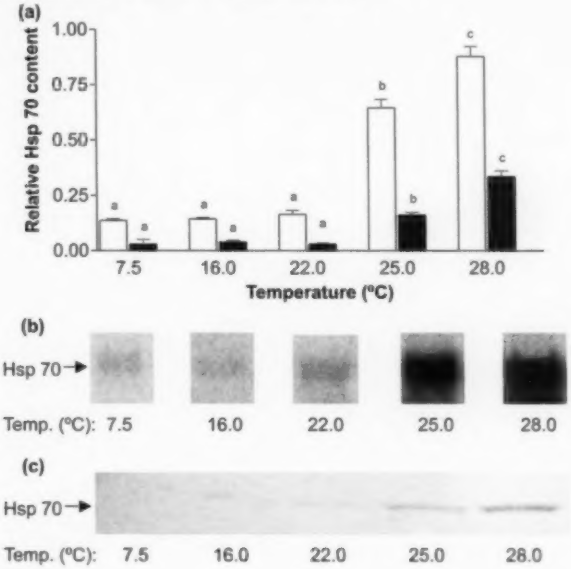
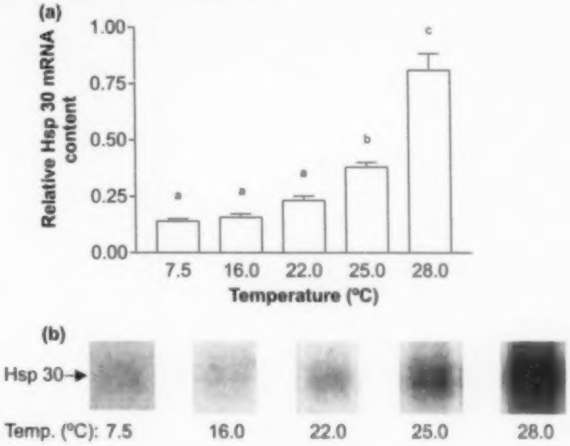


Fig. 2. (*a*) Relative mRNA concentrations of heat-shock protein 30 (Hsp 30) in whole body torsos ($N = 10$ for 7.5°C controls; $N = 4$ for all other temperatures) from Atlantic salmon (*Salmo salar*) parr exposed to an acute temperature stress under experimental conditions. Differing letters denote a significant difference within mRNA samples ($P \le 0.05$). All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern bands for Hsp 30 mRNA induced in Atlantic salmon torsos as described in Fig. 2*a*.



samples were not simply a result of general temperature effects on the synthesis of all proteins, this blot was also probed with α-tubulin. This analysis showed that there were no significant differences in α-tubulin mRNA content between any of the conditions examined (data not shown). The same pattern of protein synthesis observed for Hsp 70 was also observed for Hsc 70. Once again, there were no significant differences between the field sample periods for Catamaran Brook, but there was a significant increase in Hsc 70 protein content during the high-temperature event in the LSW. Hsc 70 protein content was also significantly higher in field samples at 27°C than in laboratory heat-shocked fish (28°C).

It is noteworthy that, in some cases, immunodetection of Hsp or Hsc 70 protein levels resulted in two stacked bands in the 70 kDa range. Under these circumstances, both bands were quantified together because they reacted similarly to our antibodies. For Hsc 70, this phenomenon was only observed in the four parr sampled at 27°C in the LSW. It was also observed for Hsp 70, however, in all four parr sampled at both 20°C and 27°C in the LSW and in one and two parr sampled from Catamaran Brook during the late spring and high-temperature conditions, respectively. At this point, the exact reason for the appearance of this double band is unclear. Although it is conceivable that the additional band in some of our field samples may represent another Hsp isoform in Atlantic salmon that is recognized by our Hsp antibodies, further investigations in this area are warranted before this issue can be fully resolved. In regard to this issue, it is also important to note, however, that the observed changes in Hsp protein levels within this study are strongly supported by changes in Hsp mRNA. Thus, the appearance of this double band within some of our field samples does not significantly influence the major findings within this study.

Field temperature measurements

The number of days in which air temperature exceeded 25°C at Catamaran Brook was highest in 1999, followed closely by 2001 (Table 1). High air temperatures had the most significant warming effect on water within the larger of the two rivers, the LSW Miramichi River. Water temperatures exceeded 23°C in this river for 62 and 52 days in 1999 and 2001, respectively. It is also noteworthy that the lower reach of Catamaran Brook, closest to the confluence of the LSW Miramichi River, experienced more high temperature days than did the middle reach. Despite the fact that air temperatures routinely exceeded 25°C at the Catamaran Brook in the summers of 1999 and 2001, water temperatures in the middle reach of this brook only exceeded 23°C on four days in 1999 and one day in 2001.

Diurnal water temperature variations recorded from day 213 (1 August 2001) until day 223 (11 August 2001) indicate that the duration of warm-water events can be variable, lasting anywhere from a few hours to a few days (Fig. 7). For instance, at the end of day 214 (2 August 2001), air temperature reached 32.8°C with water temperatures reaching 27.4°C in the Little Southwest Miramichi River and 21.5°C in Catamaran Brook. On the following day (3 August 2001;

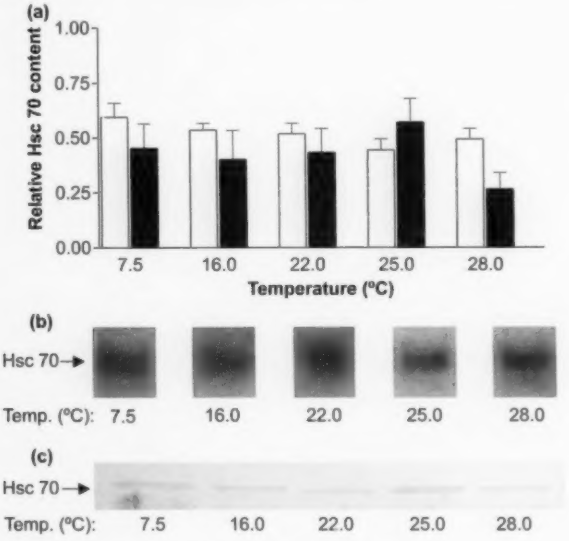


Fig. 3. (*a*) Relative concentrations of heat-shock cognate 70 (Hsc 70) mRNA (open bars) and protein (solid bars) in whole body torsos ($N = 10$ for 7.5°C controls; $N = 4$ for all other temperatures (mRNA); and $N = 4$ for all protein samples) from Atlantic salmon (*Salmo salar*) parr exposed to an acute temperature stress under experimental conditions. All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern bands for Hsc 70 mRNA induced in Atlantic salmon torsos as described in Fig. 3*a*. (*c*) Representative western bands for Hsc 70 protein levels in Atlantic salmon torsos as described in Fig. 3*a*.

high-temperature sampling day for series II), although maximum air temperatures decreased significantly to 28.5°C, maximum water temperatures in both rivers increased (Catamaran Brook, 22.7°C; LSW Miramichi River, 27.5°C). By 6–7 August 2001 (day 218 to day 219), water temperatures in the Little Southwest Miramichi River exceeded 23°C for the greater part of each day. Although this prolonged daily warming was not observed in Catamaran Brook, peak daily temperatures did frequently reach 23°C within this smaller stream.

Discussion

The most candid evidence of heat-induced protein damage within organisms is the appearance of elevated levels of heat-shock mRNAs and their respective proteins (Lindquist and Craig 1988; Parsell and Lindquist 1993). Despite the vast amount of literature published each year on the heat-shock response, however, there is a paucity of information regarding hsp synthesis in organisms exposed to heat stresses that would be encountered in their natural environment. Because poikilothermic organisms such as fish have body temperatures that are normally within 1°C of their aqueous surroundings (Reynolds et al. 1976), they are extremely sensitive to changes in water temperature and, in this respect, may be excellent models on which to study the sublethal effects of environmental heat stress.

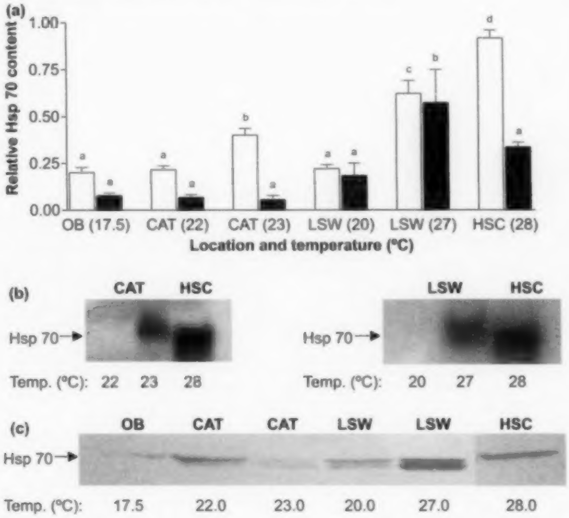


Fig. 4. (*a*) Relative mRNA (open bars) and protein concentrations (solid bars) of Hsp 70 in whole body torsos ($N = 7$ for field samples; $N = 4$ for heat-shock controls (mRNA); and $N = 4$ for all protein samples) from Atlantic salmon (*Salmo salar*) parr sampled in the field. Abbreviations: Otter Brook (OB), Catamaran Brook (CAT), and Little Southwest Miramichi River (LSW). Heat-shock controls (HSC) from the laboratory experiments (28°C; Figs. 1–3) were included within the northern and western analyses for the field samples. Differing letters denote a significant difference within mRNA and protein samples, respectively ($P \leq 0.05$). All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern blot for Hsp 70 mRNA levels in salmon torsos from CAT and LSW in comparison with HSC as described in Fig. 4*a*. (*c*) Representative western blot for Hsp 70 protein levels in salmon torsos from CAT and LSW in comparison with HSC as described in Fig. 4*a*.

Analysis of temperature data collected in the present study indicates that Atlantic salmon in the Miramichi River system may have to cope with summer water temperatures that frequently exceed 23°C and often approach their upper lethal temperature limit. Juvenile Atlantic salmon have a preference for cool to moderate water temperatures in the range of 9 to 19°C and have been shown to exhibit maximal growth at temperatures in the range of 16 to 19°C (Peterson and Martin-Robichaud 1989; Wankowski and Thorpe 1989). Elliot (1991) showed that Atlantic salmon parr could survive for more than 7 days at a maximum temperature of 27.8°C but feeding ceased at an upper temperature of approximately 22°C. An examination of the induction profile of Hsp 70 mRNA and protein in salmon parr under controlled laboratory conditions indicates that both mRNA and protein are significantly induced somewhere between 22°C and 25°C, just above the temperature at which parr stop feeding. This was also observed for Hsp 30 mRNA, which was significantly induced at about the same temperature threshold. These results support the view that threshold induction temperatures of hsps generally fall within 5–10°C above the normal physiological temperatures in which organisms live (Lindquist 1986; Parsell and Lindquist 1993; Feder and

Fig. 5. (*a*) Relative mRNA concentrations of Hsp 30 in whole body torsos ($N = 7$ for field samples; $N = 4$ for heat shock controls) from Atlantic salmon (*Salmo salar*) parr sampled in the field. Abbreviations: Otter Brook (OB), Catamaran Brook (CAT), and Little Southwest Miramichi River (LSW). Heat-shock controls (HSC) from the laboratory experiments (28°C; Figs. 1–3) were included within the northern analyses for the field samples. Differing letters denote a significant difference within mRNA samples ($P \leq 0.05$). All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern blot for Hsp 30 mRNA levels in salmon torsos from CAT and LSW in comparison with HSC as described in Fig. 5*a*.

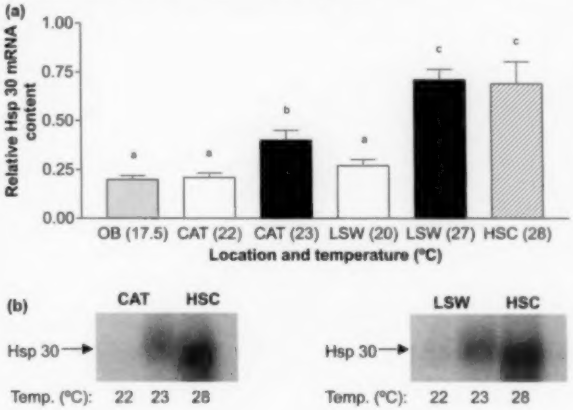
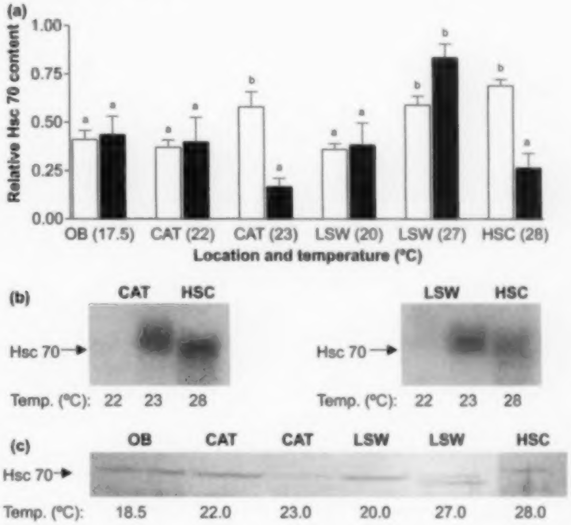


Fig. 6. (*a*) Relative concentrations of heat-shock cognate (Hsc 70) 70 mRNA (open bars) and protein (solid bars) in whole body torsos ($N = 7$ for field samples; $N = 4$ for heat shock controls (mRNA); and $N = 4$ for all protein samples) from Atlantic salmon (*Salmo salar*) parr sampled in the field. Abbreviations: Otter Brook (OB), Catamaran Brook (CAT), and Little Southwest Miramichi River (LSW). Heat-shock controls (HSC) from the laboratory experiments (28°C; Figs. 1–3) were included within the northern and western analyses for the field samples. Differing letters denote a significant difference within mRNA and protein samples, respectively ($P \leq 0.05$). All values are expressed as mean ± 1 standard error of the mean. (*b*) Representative northern blot for Hsc 70 induced in salmon torsos from CAT and LSW in comparison with HSC as described in Fig. 6*a*. (*c*) Representative western blot for Hsc 70 induced in salmon torsos from CAT and LSW in comparison with HSC as described in Fig. 6*a*.



Hofmann 1999). Although there have been no previous studies examining the heat-shock response in Atlantic salmon in vivo, Smith et al. (1999) showed that proteins in the 65 to 66 kDa range were significantly induced in isolated branchial lamellae and erythrocytes in vitro by temperatures as low as 20°C.

Hsp 70 and Hsp 30 mRNA levels measured in Atlantic salmon parr sampled in the field from Catamaran Brook and the Little Southwest Miramichi River were significantly higher during the summer warming event in August than during late spring control conditions in June. The levels of both Hsp 70 and Hsp 30 mRNA in Atlantic salmon parr obtained for all three rivers, during both late spring control and heat stress conditions, also correlate well with levels recorded over a similar temperature range under laboratory conditions. The field results indicate that 23°C is probably the threshold for mRNA induction for both of these hsps. In contrast to the situation for Hsp 70 mRNA levels, the threshold for significant increases in Hsp 70 protein appears to occur at a temperature that is somewhat higher than 23°C. Although our laboratory experiments indicated that the induction threshold for Hsp 70 protein also falls between 22°C and 25°C, Hsp 70 protein levels were not significantly increased in the field at 23°C. Hsp 70 protein levels in the field were therefore only significantly elevated in Atlantic salmon parr from the Little Southwest Miramichi River under heat stress conditions at 27°C. Although the mRNA and protein results for Hsp 70 induction may appear to be somewhat conflicting, they can probably be largely attributed to the fact that changes in hsp mRNA levels are a more sensitive indicator of thermal stress (Lis and Wu 1994). Because denaturation and aggregation of proteins is believed to be the primary signal for hsp mRNA induction (Hightower 1980; Anathan et al. 1986; Morimoto 1993), our combined results can be viewed as strong evidence that Miramichi River Atlantic salmon parr are probably experiencing significant protein damage in the wild when water temperatures exceed 23°C. In this regard, it is noteworthy that water temperature in the Little Southwest Miramichi River exceeded 23°C for an average of 29 days each summer over the past 10 years. To our knowledge, the effects of these temperature profiles on the growth and development of Miramichi Atlantic salmon parr have not been documented. Our findings suggest, however, that this issue clearly warrants further investigation because the induction of an hsp response has been shown to suppress the synthesis of other proteins (Parsell and Lindquist 1993). Future studies in this area should probably also monitor the levels of ubiquitin-conjugated proteins to gain insight into the levels of irreversible protein damage that occurs during these summer warming events.

Unlike the inducible stress proteins examined in the present study, constitutive Hsc 70 mRNA or protein was not

Fig. 7. Diurnal water temperature variation for Catamaran Brook middle reach (shaded line) and the Little Southwest Miramichi (heavy solid line) vs. air temperature (dashed–dotted line) from day 213 (Aug. 1) until day 223 (Aug. 11), 2001. Day 215 was the high-temperature sampling day. Lines represent hourly (GMT) recorded water and air temperatures as described in Materials and methods. As a reference, the horizontal solid line running through the graph indicates a temperature of 23°C.

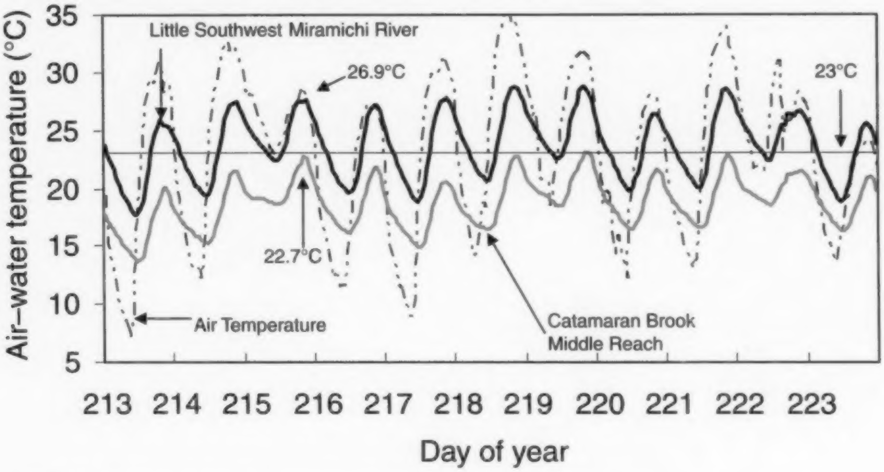


Table 1. Number of days with stream water temperature (WT) exceeding 23°C in Catamaran Brook (Middle and Lower Reach) and Little Southwest Miramichi River (NB) and number of days with air temperature (AT) exceeding 25°C at Catamaran Brook.

Year	Catamaran Brook (Middle Reach), WT > 23°C	Catamaran Brook (Lower Reach), WT > 23°C	Little Southwest Miramichi River, WT > 23°C	Catamaran Brook AT > 25°C
1992	0	0	8	28
1993	0	0	17	39
1994	0	12	30	46
1995	0	19	57	52
1996	0	0	19	29
1997	0	0	14	29
1998	0	6	16	47
1999	4	41	62	68
2000	0	13	19	31
2001	1	25	52	54

induced in Atlantic salmon parr during heat shock under laboratory conditions. This would be expected, as constitutive members of the Hsp 70 family are thought to be primarily responsible for "molecular chaperoning" activities during normal cellular conditions and are not generally induced following heat stress (Georgopoulos and Welch 1993; Hendrick and Hartl 1993). Several previous studies examining the effects of heat shock on Hsc 70 expression in fish have been carried out on isolated tissues or cell lines (Zafarullah et al. 1992; Arai et al. 1994; White et al. 1994), and only one of these studies showed a slight induction of Hsc 70 protein in response to heat stress (Arai et al. 1994). In contrast to the laboratory data obtained in the present study, however, Atlantic salmon parr sampled during the high-temperature event in both the CAT and LSW contained significantly more Hsc 70 mRNA than did parr sampled during the control conditions in the spring. The induction of Hsc 70 mRNA was paralleled by an increase in Hsc 70 protein in the LSW during the warm event at 27°C, but not at 23°C in Catamaran Brook. Although previous studies have shown that large-scale variations in Hsp 70 content may occur within organisms as a result of extraneous factors such as species location or seasonal temperature variation (Ulmasov et al. 1992; Fader et al. 1994), no similar studies have been done on the constitutive Hsc 70 protein. Nonetheless, the present results suggest that chronically elevated temperatures or other seasonal factors in the wild may be influencing the Hsc 70 protein and mRNA levels in salmon parr from both Catamaran Brook and the Little Southwest Miramichi River. Further analysis of α-tubulin levels indicated that the relative increase in Hsc 70 mRNA content observed between fish sampled during the spring and the late summer warming event was not simply due to a general temperature effect on the synthesis of all proteins. One can speculate, however, that increased levels of protein synthesis and growth during the warm summer months could necessitate higher levels of constitutive hsps in order to provide adequate chaperone functions for nascent polypeptide folding and translocation. Regardless, the present results show that the levels of Hsc 70 may vary within wild populations, and further investigations in this area are probably warranted to determine the exact reasons for these changes.

In summary, the combination of heat-shock mRNA and protein induction profiles obtained from both laboratory experiments and field sampling, as well as river temperature records, in the present study clearly show that Miramichi Atlantic salmon parr are commonly exposed to significant heat stress during the summer months. These results provide further evidence that the hsp response does commonly occur in some wild populations. Although this hsp response is an important adaptation contributing to the survival of organisms under extreme environmental conditions, it can also be viewed as an "early warning" that a given population may be experiencing significant sublethal thermal stress. In this regard, our findings also suggest that any further increases in water temperatures as a result of climate change could have profound consequences for one of Canada's most productive Atlantic salmon rivers.

Acknowledgments

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Functional plasticity of benthic macroinvertebrates: implications for trophic dynamics in acid streams

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Abstract: Functional plasticity of benthic macroinvertebrates was investigated over one year in four acid streams in the Vosges Mountains (northeastern France). The trophic position of macroinvertebrate species within the benthic food web was determined using gut content analyses. Diet analyses revealed that only 24–36% of biomass of putative shredders consumed leaf fragments, whereas up to 44% consumed benthic algae and bryophytes. Although most Nemouromorpha stoneflies were generalist consumers, several other taxa (e.g., *Brachyptera seticornis*, *Chaetopterygopsis maclachlani*) specialised on benthic algae and bryophytes. Our study showed that acid streams unexpectedly had very few specialised leaf-shredding species (e.g., *Chaetopteryx villosa*) that could explain the slow leaf detritus processing rates observed in these systems. Primary producers appear to be an alternative resource for shredders, playing an important role in supporting food webs in forested acid streams. The food web built in this study suggests that overlooking species-specific functional plasticity of invertebrates may result in a misconception of invertebrate community structure in acid streams.

Résumé : La variabilité du rôle fonctionel trophique des macroinvertébrés benthiques a été étudiée pendant une année dans quatre ruisseaux acidifiés du Massif Vosgien (Nord-Est de la France). Le rôle des différents taxons de macroinvertébrés benthiques au sein du réseau trophique lotique a été déterminé par des analyses de leurs contenus digestifs. Les résultats montrent qu'en terme de biomasse, seulement 24 % à 36 % des invertébrés *a priori* considérés comme déchiqueteurs se nourrissent effectivement de litières en décomposition, alors qu'une majorité d'entre eux (jusqu'à 44 %) consomment des algues benthiques et des bryophytes. Bien que la plupart des plécoptères Neroumorpha possède un régime alimentaire généraliste, certaines espèces (e.g., *Brachyptera seticornis*, *Chaetopterygopsis machlachlani*) sont spécialisées dans la consommation d'algues benthiques ou de bryophytes. Contrairement à ce qui est généralement admis, il semble donc que les ruisseaux acidifiés abritent en fait très peu d'espèces de déchiqueteurs (e.g., *Chaetopteryx villosa*), expliquant ainsi la lente décomposition des litières observée dans ces systèmes. Consommés par un grand nombre de déchiqueteurs, les producteurs primaires semblent jouer un rôle important dans les réseaux trophiques des ruisseaux acidifiés. Par ailleurs, cette étude suggère certaines limites de l'utilisation des groupes fonctionels trophiques dans l'étude du fonctionnement des écosystèmes aquatiques perturbés.

Introduction

Recently, acidic deposition has resurfaced as an international environmental issue (Driscoll et al. 2001). Acidified streams are biologically impoverished systems owing to the adverse impacts of multiple stressors (e.g., proton and aluminum excess, calcium deficiency, and increased exposure to ultraviolet radiation) (see Brakke et al. 1994). Leaf-shredding invertebrates, mainly filipalpian stoneflies, are abundant (Sutcliffe and Hildrew 1989), but leaf decomposition is slow in acidified streams (Griffith and Perry 1993; Dangles and Guérold 2001*a*). Both bottom-up and top-down controls have been proposed to explain such contradictory patterns. Acidification can affect microbial decomposition and increase the quantity of food available to shredders, even though its quality is low (Townsend and Hildrew 1984). On the other hand, slower breakdown rates of litter in acidic streams compared with neutral ones may be related to taxonomic differences in the shredder assemblages, rather than differences in shredder abundance and (or) biomass (Griffith and Perry 1993; Dangles and Guérold 2001*a*). This latter assertion supposes that the functional role played by some acid-sensitive shredders species in neutral streams might not be fulfilled by other shredder species in acidified streams (Dangles and Guérold 2001*b*).

Leaf litter breakdown is a key function of headwater-forested streams located in poorly acid-buffered catchments. Allochthonous inputs, especially litter from riparian trees, have been shown to regulate secondary production and the food web structure of these streams (Wallace et al. 1997). Invertebrate shredders often play crucial roles in foliar allochthonous carbon assimilation, production of fine particulate organic matter, and organic matter cycling in lotic systems (Wallace and Webster 1996). As a result, their role in

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stream ecosystem function has been a central research area for stream ecologists for the last 30 years. Several taxa have been widely recognized to have evolved leaf shredding strategies, such as decapod and amphipod Crustacea and nemourid, limnephilid, and tipulid Insecta (see Graça 2001 for review). Although the literature has shown that trophic niches of benthic invertebrates may vary among habitat, season, or ontogeny (e.g., Moore 1977; Malmqvist et al. 1991), the ascribed function of a given taxon has often been too general to answer specific process-oriented questions. Even though many studies have stressed the danger of using preassigned trophic groups (e.g., Minshall 1988; MacNeil et al. 1997; Mihuc 1997), little attention has been paid to the implications this can have on our perception of ecosystem processes.

In this paper, I hypothesize that the ascribed function of stream invertebrate species, especially shredding, may be flexible (both in diet and mode of feeding) and subject to local conditions. The main objective of my study was to test this hypothesis by investigating the functions of invertebrate species in streams disturbed by anthropogenic acidification. The functions were determined by identifying the position of invertebrate species within the benthic food web using gut content analyses. I focused on putative shredders because of their high abundance and potentially strong effect on leaf litter decomposition in acid streams. Furthermore, I evaluated the accuracy of functional feeding group (FFG) assignments for the understanding of processes, food webs, and restoration of acid streams. Although it has already been stated that inaccurate characterisation of the trophic function of invertebrates may lead to misunderstanding of the overall ecosystem function (Minshall 1988; Mihuc 1997), little evidence has been presented to substantiate the argument.

Materials and methods

Site description

The study was conducted in four first- and second-order acid streams in the Vosges Mountains (northeastern France). All the streams drained sandstone or granitic forested catchments dominated by silver fir (*Abies alba* Karst.), Norway spruce (*Picea abies* Karst.), and common beech (*Fagus sylvatica* L.), the most common riparian deciduous tree species providing the bulk of particulate organic matter inputs. The four streams, Gentil Sapin (GS), Wassongoutte (WA), Bas-Rupts (BR), and Rouge-Rupts (RR), had very similar geomorphologic characteristics, especially in terms of water depth (≤0.70 m), stream width (≤4.50 m), and gradient (>8%) (Table 1). Standing stock of coarse particulate organic matter ranged from 45.3 $mg \cdot m^{-2}$ (GS) to 75.5 $mg \cdot m^{-2}$ (BR). All streams were located upstream of anthropogenic activities such as housing, farming, and industrial activities (except selective logging).

Habitat availability within the streams was determined at each of the four sites by assessing about 50 1-m^2 quadrats in a 20-m section (see Dangles 2000 for more details). Benthic habitats in the selected reaches included riffles, bryophyte-covered bedrock, and depositional areas formed behind natural channel obstructions like debris dams (Table 1). Streams at the four sites were predominantly cobble and boulder riffles, with the proportion of depositional areas ranging from 14.8 (WA) to 22.5% (GS).

All the selected streams had low pH (4.6–5.1) as a result of high acid deposition in the region during the last decades (Dambrine et al. 1998). They presented typical chemical patterns of acid-stressed headwaters, i.e., low conductivity, negative alkalinity, and elevated aluminium (Al) concentrations. Water temperature varied between 0 and 10°C with minima in February and maxima in September (Table 1). More details on the water chemistry of the streams are given in Dangles and Guérold (2001*a*). The four streams were used as replicates to integrate spatial variability and to strengthen conclusions drawn from the results.

Benthic sampling

Macroinvertebrate collections were made on seven occasions between November 1997 and September 1998. Surber samples (0.072 m^2, 0.28-mm mesh size) were taken from five habitat types: pebble–gravel run, cobble–boulders, bryophyte-covered bedrock, and sand and detrital pools (Table 1). In areas of unconsolidated substrata (depositional areas), the stream bed was sampled to a depth of ~5 cm. Samples were fixed in 5% formaldehyde in the field, and then in the laboratory the invertebrates were sorted and identified to the lowest feasible taxonomic level (mainly species and genus), except for early instars of Limnephilinae, Chironomidae (family level), and Oligochaeta. To verify species identification, we also sampled adult insects by sweep-netting the riparian vegetation. Following identification, we sorted and enumerated individuals of each taxon into approximate size-classes (small, medium, large). Biomass was determined by weighing dried (48 h at 60°C) individuals to the nearest 0.01 mg. The relative areas of streambed composing each of the five habitats patch types were used to adjust density, abundance, and biomass data.

Diet analysis

The study focused on organisms usually described as shredders (abbreviated P-shredders, putative shredders, in the rest of the text) in the literature (Cummins 1973; Merritt and Cummins 1984; Tachet et al. 1987). The composition of invertebrate diets was determined by gut content analysis using a modification of techniques described by Shapas and Hilsenhoff (1976). The number of individuals of each taxon analysed on each sampling date varied between 1 and 15 depending on their abundance. Teased-out foregut contents were placed in a drop of water on a microscope slide. Ten randomly selected fields were viewed at 100× magnification. Six food categories were identified: (*i*) animal matter, (*ii*) fine amorphous detritus, (*iii*) diatoms, (*iv*) filamentous algae, (*v*) coarse leaf detritus, and (*vi*) particles derived from bryophytes. Fine amorphous detritus was distinguished from other items by its lack of well-defined cellular structure. Coarse leaf detritus was identified by the presence of brownish cells and palisade cell layers. Complementary studies of the gut contents of several P-shredder taxa (*Leuctra*, *Protonemura*, *Sericostoma personatum*, *Chaetopteryx villosa*) fed leaves under laboratory conditions aided in the recognition of leaf material. The approximate percentage by area of the six food items was recorded in each field on the slide.

Table 1. Description of the study streams (GS, Gentil Sapin; WA, Wassongoutte; BR, Bas-Rupts; RR, Rouge-Rupts).

	GS	WA	BR	RR
Geomorphic features				
Bedrock	Sandstone	Granite	Granite	Granite
Order	1	1	1	2
Altitude (m)	510	690	845	675
Catchment area (km^2)	4.65	1.32	0.68	9.65
Distance from source (m)	3100	2600	700	4700
Water depth (m)	0.50	0.45	0.40	0.70
Stream width (m)	2.30	2.00	1.40	4.50
Gradient (%)	10	13.8	20	8.4
Habitat proportions (%)				
Riffles				
Pebble–gravel run	16.1	14.2	12.9	10.3
Cobble–boulders	43.6	61.6	55.0	60.8
Bryophyte-covered bedrock	17.8	9.4	11.0	9.0
Depositional areas				
Sand	10.3	7.6	13.8	14.8
Detrital pools	12.2	7.2	7.3	5.0
Physico-chemical factors				
pH^a	4.6 (4.5–4.9)	4.9 (4.7–5.7)	5.1 (4.9–5.6)	5.0 (4.9–5.8)
Conductivity (μS·cm^{-1}, 20°C)	35	17	21	17
Alkalinity (μeq·L^{-1})	–19	–6	–3	–2
Total aluminum (μg·L^{-1})	802	295	280	255
Temperature (°C)	2.1–10	0–10	0–10	0–10
CPOM standing stock (mg·m^{-2})	45.3	60.1	75.5	62.6

Note: Water depth and stream width are mean values for each station. Coarse particulate organic matter (CPOM) standing stock values are mean ash-free dry biomass (n = 7) adjusted for habitat availability. Temperatures are minimum and maximum values and pH, alkalinity, and total aluminum are means (n = 8) for the study period.

[a] Range for pH is given in parentheses.

Diets of other abundant macroinvertebrate taxa (including predators and collectors) were also investigated using the same protocol to build food web diagrams. Prey items found in the foreguts of predator taxa were identified to the lowest practicable taxonomic level. This was impossible for some taxa (e.g., *Rhyacophila*, *Polycelis felina* Dalyell, *Pedicia*) because of excessive prey maceration or because they are sucking predators.

Statistical methods

To investigate spatial, temporal, and ontogenic variability in the diet of common taxa, gut contents were analysed with regard to stream, date (four seasons), and larval size. Arcsine-transformed percentages of each major food type (see above) were used to perform a principal component analysis (PCA). Subsequently, a three-way analysis of variance (ANOVA) using PCA scores for each taxon was used to compare the importance of size, stream, and date. However, this analysis was only acceptable for *Protonemura* and *Leuctra*, as there were insufficient numbers of animals collected for most other taxa.

To summarize the complexity of food webs in acid streams, I collapsed macroinvertebrate taxa into trophic species (sensu Cohen et al. 1990) using cluster analysis. The use of the term "species" in this context does not imply a biological species, but rather a collection of organisms that have the same diets and the same predators, and thus perform a similar function in the system. For the cluster analysis, only the most abundant taxa found in the four streams (both in terms of abundance and biomass) were aggregated into trophic species on the basis of diet similarity. All statistical analyses were performed using Statistica v. 5.5 (StatSoft, Inc., Tulsa, Okla.).

Results

Abundance and biomass of P-shredders

At least 29 species of P-shredders were collected from the four streams over the sampling period (see Appendix A). Of these, larvae of *Leuctra*, *Protonemura*, and *Nemoura* could not be identified to species level because of high diversity in the regional species pool (7 species, 8 species, and 12 species, respectively). These three genera were treated as single taxa in all analyses. Thirteen taxa made up more than 95% of the density and biomass of P-shredders (Table 2). Mean P-shredder density varied between 807 individuals·m^{-2} (BR) and 1215 individuals·m^{-2} (RR). Numerically, the P-shredder populations were dominated by nemourid and leuctrid Plecoptera, especially *Protonemura* and *Leuctra*, which accounted for 52.6 (RR) to 87.2% (GS) of total abundance, respectively. Total biomass of P-shredders ranged from 129 mg·m^{-2} (GS) to 260 mg·m^{-2} (WA). Biomass was dominated by Plecoptera (*Protonemura* and *Leuctra*), but some species of Trichoptera (especially *C. villosa* and *Chaetopterygopsis maclachlani*) occasionally made a substantial contribution to total P-shredder biomass.

Table 2. Mean density (*D*, individuals·m^{-2}) and mean biomass (dry mass; *B*, mg·m^{-2}) of putative shredders in the four streams (GS, Gentil Sapin; WA, Wassongoutte; BR, Bas-Rupts; RR, Rouge-Rupts), from November 1997 to September 1998.

		GS	WA	BR	RR
Plecoptera					
Brachyptera seticornis	*D*	0	131.7 (10.9)	193.6 (23.7)	234.4 (19.3)
	B	0.0	44.3 (17.0)	34.4 (14.2)	39.9 (18.5)
Protonemura	*D*	305.4 (34.0)	573.4 (47.5)	291.1 (35.6)	397.6 (32.8)
	B	29.6 (22.8)	48.0 (18.4)	44.0 (18.8)	58.6 (27.2)
Amphinemura sulcicollis	*D*	2.0 (<1)	115.6 (9.6)	24.7 (3)	134.6 (11.1)
	B	<1 (<1)	11.1 (4.3)	<1 (<1)	21.9 (10.1)
Nemoura	*D*	71.3 (7.9)	18.6 (1.5)	49.4 (6.0)	12.4 (1)
	B	5.9 (4.5)	3.4 (1.3)	3.1 (1.3)	<1 (<1)
Nemurella picteti	*D*	4.1 (0.5)	2.6 (< 1)	<1 (<1)	<1 (<1)
	B	1.6 (1.2)	1.7 (< 1)	<1 (<1)	<1 (<1)
Leuctra	*D*	418.1 (53.2)	310.0 (25.7)	139.0 (17.0)	414.1 (33.9)
	B	42.9 (33.0)	41.7 (16.0)	22.7 (9.7)	34.3 (15.9)
Total	*D*	861.0 (95.9)	1151.7 (95.3)	698.6 (85.4)	1191.0 (98.2)
	B	79.7 (61.6)	150.4 (57.8)	105.4 (45.0)	156.4 (72.1)
Trichoptera					
Drusus annulatus	*D*	2.0 (<1)	16.4 (1.4)	20.7 (2.4)	5.7 (<1)
	B	2.3 (1.8)	52.4 (20.1)	23.3 (9.9)	3.7 (1.8)
Drusus discolor	*D*	1.9 (<1)	1.1 (<1)	5.7 (<1)	0
	B	6.4 (4.9)	<1 (<1)	9.0 (3.8)	0.0
Chaetopterygopsis maclachlani (instars > III)	*D*	25.7 (2.9)	16.9 (1.4)	59.0 (7.2)	2.4 (<1)
	B	32.6 (25.1)	14.9 (5.7)	26.1 (11.1)	5.3 (2.4)
Chaetopteryx villosa (instars > III)	*D*	1.9 (<1)	11.3 (<1)	11.1 (1.4)	6.6 (<1)
	B	2.4 (1.9)	30.9 (11.9)	26.1 (11.1)	41.3 (19.2)
Limnephilinae (instars ≤ III)	*D*	4.3 (<1)	8.6 (<1)	5.4 (<1)	3.7 (<1)
	B	1.1 (<1)	1.4 (<1)	<1 (<1)	<1 (<1)
Other Trichoptera	*D*	<1 (<1)	<1 (<1)	2.7 (<1)	3.1 (<1)
	B	3.7 (2.9)	2.0 (<1)	9.0 (3.8)	8.9 (4.1)
Total	*D*	36.6 (4.1)	55.0 (4.6)	104.9 (12.8)	21.9 (1.8)
	B	48.6 (37.5)	101.6 (39.0)	115.0 (49.1)	60.0 (27.8)
Diptera					
Tipula (*Saginata*)	*D*	<1 (<1)	<1 (<1)	<1 (<1)	<1 (<1)
	B	<1 (<1)	<1 (<1)	<1 (<1)	<1 (<1)
Tipula (*Savtschenkia*)	*D*	<1 (<1)	1.1 (<1)	2.4 (<1)	0.0
	B	1.0 (<1)	7.9 (3.0)	13.4 (5.7)	0.0
Total	*D*	<1 (<1)	1.3 (<1)	2.6 (< 1)	<1 (<1)
	B	1.1 (<1)	8.0 (3.0)	13.6 (6.0)	<1 (<1)

Note: Values in parentheses are percent contributions of taxa to total shredder density and biomass. "Other Trichoptera" includes *Crunoecia irrorata*, *Anomalopterygella chauviniana*, *Acrophylax zerberus*, *Micropterna lateralis*, *Potamophylax cingulatus*, *Allogamus uncatus*, and *Sericostoma personatum*.

Gut content analysis of shredding invertebrates

Foregut content analyses showed that coarse leaf detritus and fine amorphous detritus were the predominant foods of most P-shredder taxa (Table 3). However, aquatic algae and bryophytes were major components of the diet of several species. *Brachyptera seticornis*, *Drusus annulatus*, and *Anomalopterygella chauviniana* fed mainly on benthic algae. *Chaetopterygopsis maclachlani* and *Tipula* (*Savtchenkia*) were bryophyte specialists. According to their gut contents, *Leuctra* and *Protonemura*, by far the most abundant taxa, had generalist feeding habits. No significant relationships between food types and season or streams were found for these two genera (three-way ANOVA, $F_{3,3,2} < 2.17$, $P > 0.05$). However, early instars of both *Leuctra* and *Protonemura* had significantly more fine amorphous detritus and benthic algae in their guts than larger individuals (three-way ANOVA, $F_{3,3,2} = 5.05$, $P < 0.01$).

By combining diet analyses with animal biomass, it was possible to investigate temporal variation in food resource use by P-shredders in the four streams (Fig. 1). The number of P-shredders feeding on coarse leaf detritus was low throughout the year (Fig. 1*a*). However, biomass of P-shredders containing leaf fragments was high in late summer samples, mainly because late instars of *C. villosa* were present (Fig. 1*e*). Abundance and biomass of putative P-shredders consuming fine amorphous detritus were generally high and showed no seasonal trends (Figs. 1*b* and 1*f*). Benthic algae were also consumed by numerous P-shredders,

Table 3. Annual mean percent abundance of each food category ingested by putative shredders in the four streams.

	No. individual analyses	Coarse leaf detritus	Amorphous fine detritus	Diatoms	Filamentous algae	Bryophytes	Animal matter
Brachyptera seticornis	46	0.0 (0)	24.6 (25.8)	26.8 (22.5)	48.6 (26.5)	0.0 (0)	0.0 (0)
Protonemura	175	30.5 (21.1)	43.8 (26.2)	20.6 (21.3)	3.3 (16.8)	1.8 (15.8)	0.0 (0)
Amphinemura sulcicollis	33	11.4 (38.2)	50.7 (37.1)	9.3 (28.8)	28.6 (32.5)	0.0 (0)	0.0 (0)
Nemoura	32	21.4 (29.5)	44.8 (29.8)	15.4 (22.3)	13.4 (28.2)	0.0 (0)	5.0 (23.9)
Nemurella picteti	15	41.2 (12.6)	11.6 (14.2)	13.3 (10.6)	33.9 (16.2)	0.0 (0)	0.0 (0)
Leuctra	220	21.3 (18.6)	50.4 (22.9)	14.9 (15.5)	12.3 (19.6)	1.1 (18.2)	0.0 (0)
Crunoecia irrorata	8	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Drusus annulatus	50	0.0 (0)	30.7 (35.5)	49.4 (39.5)	19.9 (30.1)	0.0 (0)	0.0 (0)
Drusus discolor	43	4.6 (15.2)	14.8 (11.9)	32.4 (13.9)	11.1 (19.6)	0.0 (0)	37.1 (18.6)
Anomalopterygella chauviniana	4	0.0 (0)	22.2 (11.2)	58.1 (17.5)	19.7 (6.5)	0.0 (0)	0.0 (0)
Acrophylax zerberus	4	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Micropterna lateralis	5	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Potamophylax cingulatus	27	89.3 (8.5)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	10.7 (8.5)
Allogamus uncatus	3	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Chaetopterygopsis maclachlani	75	35.5 (8.3)	0.0 (0)	0.0 (0)	0.0 (0)	64.5 (8.3)	0.0 (0)
Chaetopteryx villosa	45	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Limnephilinae (instars ≤ III)	26	38.8 (15.5)	29.9 (20.3)	21.2 (25.2)	10.1 (21.9)	0.0 (0)	0.0 (0)
Sericostoma personatum	5	100 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Tipula (*Saginata*)	10	71.2 (14.5)	0.0 (0)	0.0 (0)	9.1 (16.5)	0.0 (0)	19.7 (14.2)
Tipula (*Savtchenkia*)	37	4.4 (5.2)	0.0 (0)	0.0 (0)	0.0 (0)	95.6 (5.2)	0.0 (0)

Note: Standard deviation is given in parentheses.

mainly during winter (Fig. 1*c*). However, the temporal peak was not as apparent when considering biomass (Fig. 1*g*). Bryophytes were ingested by a small number of individuals throughout the year (Fig. 1*d*), and a few individuals (mainly late instars of *C. maclachlani* and *Tipula* (*Savtchenkia*)) had ingested a large amount of this material, especially in BR (Fig. 1*h*).

In summary, only 23.8 (WA) to 35.6% (BR) of P-shredders, in terms of biomass, fed on leaf detritus over the year (Table 4). Up to 44.7% (WA) of the total biomass of P-shredders fed on benthic algae and bryophytes. The relative proportions of the different food items ingested remained relatively constant among the four replicate streams, except for bryophytes, which were infrequently eaten in RR.

Table 4. Mean annual percentage of the biomass of putative shredders consuming the different food resources (leaf fragments, amorphous detritus, diatoms, filamentous algae, bryophytes, animal matter) in the four study streams (GS, Gentil Sapin; WA, Wassongoutte; BR, Bas-Rupts; RR, Rouge-Rupts).

	GS	WA	BR	RR
Coarse leaf detritus	27.1	23.8	35.6	34
Amorphous fine detritus	33.6	31.5	22.4	33.2
Diatoms	13.0	21.3	15.3	15.0
Filamentous algae	6.7	16.4	11.4	15.3
Bryophytes	17.5	7.0	12.8	2.2
Animal matter	2.1	0.1	2.5	0.3

Food webs

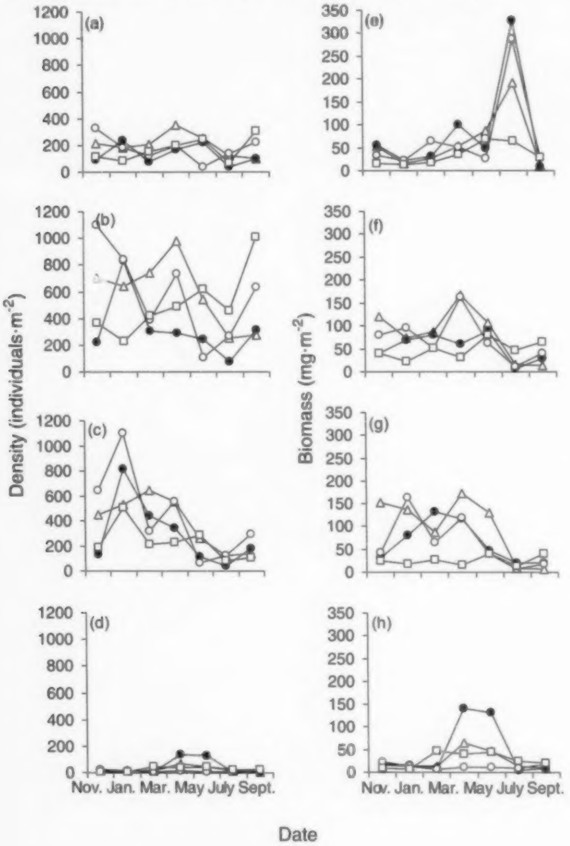
Since feeding habits of benthic macroinvertebrates did not show any significant differences among streams (one-way ANOVA, $F_3 = 0.32$, $P = 0.42$), the results of gut content analyses were pooled to build a generalised food web diagram of food web dynamics for acid streams. The cluster analysis showed that the taxa could be divided into seven main trophic species sharing more than 68% similarity (Fig. 2): specialised leaf detritus shredders (1 taxon), specialised bryophyte shredders (2 taxa), specialised predators (7 taxa), generalist predators (4 taxa), specialised collectors (5 taxa), generalist collectors–scrapers (5 taxa), and specialised scrapers (2 taxa). The trophic species structure of the four replicate study streams was used to build a conceptual model of food webs in acid streams (Fig. 3). The food web includes the 26 most abundant macroinvertebrate taxa collected in the study streams. All these taxa were present in all four streams (except *B. seticornis*, *Tipula* (*Savtchenkia*), and *Drusus discolor*, which were absent from GS, RR, and RR, respectively). Together they represented ~90% of the total annual macroinvertebrate abundance and biomass in the four streams. This food web describes four basal trophic resources for primary consumers. Note that many of these resources comprise several biological components. For example, fine amorphous detritus is likely to be a complex mixture of leaf-derived detritus, partially digested algae, biofilm, aquatic fungi, and bacteria. Macroinvertebrate taxa were classified as primary consumers (5 trophic species, 16 taxa), secondary consumers (1 trophic species, 7 taxa), and intermediate consumers, which fed on both primary resources and primary consumers.

Discussion

Putative shredder communities

Abundant species may play major roles in controlling the

Fig. 1. Temporal variation in the density (*a–d*) and biomass (*e–h*) of putative shredders feeding on various food resources (*a*, *e*: coarse leaf detritus; *b*, *f*: fine amorphous detritus; *c*, *g*: benthic algae; *d*, *h*: bryophytes) in the four replicate study streams (GS, Gentil Sapin, open square; WA, Wassongoutte, open triangle; BR, Bas-Rupts, solid circles; RR, Rouge-Rupts, open circles). Total number and biomass of putative shredders feeding on animal matter were very low and are not presented here.



rates and direction of many community and ecosystem processes (Power et al. 1996). In the four studied streams, plecopteran P-shredders largely dominated the benthic communities, and knowledge of their trophic roles appears crucial to understanding the functioning of acid streams. Although filipalpian stoneflies specialise in shredding allochthonous detritus in many streams (Wallace and Webster 1996), this activity was poorly expressed in the acid streams studied. Instead they fed to a large extent on fine amorphous detritus and benthic algae. Similar findings have also been documented for other putative shredders by several other authors (Mihuc and Minshall 1995; MacNeil et al. 1997; Mihuc 1997), although some of these studies used laboratory feeding trials that might have created conditions that do not occur under natural conditions.

Another important aspect is misclassification of taxa as leaf shredders. For example, tipulid dipterans are commonly considered obligate or specialist shredders with enzyme systems suitable for dealing with leaf litter (Anderson and Sedell 1979). My results showed strong differences in function between *Tipula* (*Saginata*) and *Tipula* (*Savtchenkia*). Even though both taxa have almost identical morphological adaptation of mouthparts and feed by tearing out bites of organic matter, the former taxon fed mainly on leaf litter and animals, whereas the latter specialised on bryophytes. Similarly, the late instars of *C. villosa* were leaf shredders, whereas those of *C. maclachlani* were bryophyte consumers.

Implications for leaf breakdown

In a parallel study, Dangles and Guérold (2001*a*) showed, by enclosing beech leaves in fine- and coarse-mesh bags, that mechanisms underlying slow leaf breakdown in acid streams (GS, RR, WA, and BR) were related to decreased macroinvertebrate activity, rather than microbial activity. When examining shredder communities, I found no significant differences in mean annual abundance or biomass of P-shredders between acid and circumneutral streams, which is inconsistent with marked differences in beech leaf breakdown rates ($k \le 0.0012$ in acid streams vs. $k = 0.0084$ in the circumneutral stream) (Table 5). However, when I considered gut contents analyses, both numbers and biomass of benthic organisms actually involved in leaf shredding were much lower than expected for the acid streams. In contrast, numbers and biomass remained quite high in the circumneutral stream. This is partly a consequence of facultative shredders (mostly filipalpians) dominating in the acidic streams and specialised shredders (mostly *S. personatum* and *C. villosa*) dominating in the circumneutral stream. High plasticity in shredding may partly explain the variation in the leaf detritus processing generally observed in acid streams.

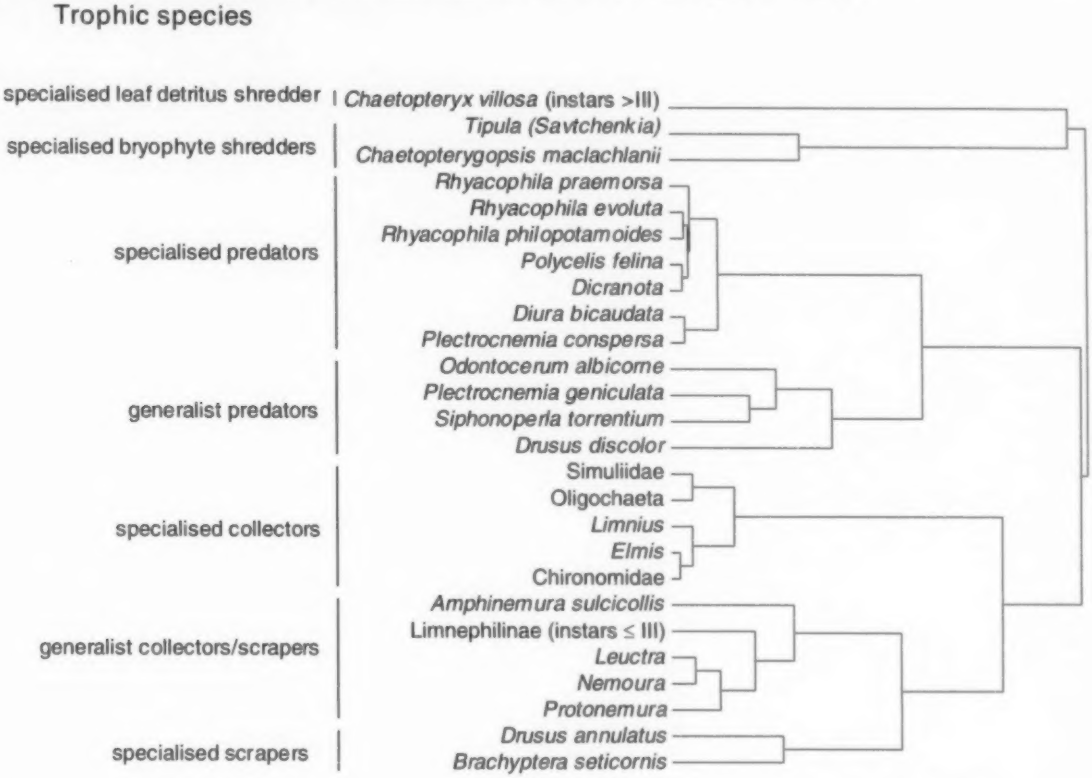
Thus, litter breakdown in acid streams appeared to depend mainly on microbial activity, and was extremely slow. As streams are open systems, it may be supposed that a large amount of the leaf detritus is not processed in headwaters, but washed downstream by flood events. Among acid tolerant shredders, *C. villosa* was likely to have the greatest impact on leaf litter breakdown. Late instars of *C. villosa* were found within detrital pools during late summer, suggesting an adaptation of its life cycle to feed on slow decomposing leaf litter. Because leaf breakdown is slow in acidic streams, the high amount of detritus available during the summer may explain why *C. villosa* is so abundant in acid streams. This is in line with the Litter Link Model devised by Cummins et al. (1989), which states that many stream detritivores have evolved life-histories with hatching and growth synchronized with the availability of microbially conditioned leaf detritus.

In this sense, the impact of *C. villosa* depended on both temporal (August) and spatial (pools) contexts, which seems to be a rule for many aquatic species (Thompson and Townsend 1999).

Use of primary production in acid streams

In many aquatic food webs, especially those of small shaded streams, such as those in the present study, detritus from surrounding vegetation typically constitutes the main food of nonpredatory species in the benthic community. Because detritus processing is slow under acidic conditions, the

Fig. 2. Cluster diagram based on the results of gut contents (complete linkage, Euclidian distance). Only the dominant taxa (representing together ~90% of the total abundance and biomass of the benthic fauna in the four streams) were selected for this analysis. All taxa grouped in the same trophic species showed more than 68% similarity in their trophic relationships.


Trophic species
specialised leaf detritus shredder
Chaetopteryx villosa (instars >III)
specialised bryophyte shredders
Tipula (Savtchenkia)
Chaetopterygopsis maclachlanii
specialised predators
Rhyacophila praemorsa
Rhyacophila evoluta
Rhyacophila philopotamoides
Polycelis felina
Dicranota
Diura bicaudata
Plectrocnemia conspersa
generalist predators
Odontocerum albicorne
Plectrocnemia geniculata
Siphonoperla torrentium
Drusus discolor
specialised collectors
Simuliidae
Oligochaeta
Limnius
Elmis
Chironomidae
generalist collectors/scrapers
Amphinemura sulcicollis
Limnephilinae (instars ≤ III)
Leuctra
Nemoura
Protonemura
specialised scrapers
Drusus annulatus
Brachyptera seticornis

possible use of alternative food resources seems likely. Our results showed that several benthic species in acid streams feed on primary producers, either sporadically (*Siphonoperla torrentium* Pictet, *Odontocerum albicorne* Scopoli, *Leuctra*, *Protonemura*) or predominantly (*B. seticornis*, *D. annulatus*).

Primary production is generally considered low in headwater streams, yet every stream tends to have a unique set of factors that control the rate of primary production (Lamberti and Steinman 1997). In acidic streams, high concentrations of atmospheric-derived nitrates in the water and a lack of acid-sensitive herbivorous invertebrates (especially mayflies) may help explain the often substantial benthic algal biomass (Mulholland et al. 1986; Winterbourn et al. 1992). Increase in inorganic carbon (H_2CO_3) availability is another important effect of water acidification. Inorganic carbon provides excess carbon to primary producers, which may lead to eutrophic conditions (see Planas 1996 for review). Autochthonous resources may play an important role in supporting food webs of forest streams (Mayer and Likens 1987; Finlay 2001). Acid streams seem to be an example of primarily heterotrophic aquatic systems in which herbivory may have become important. Modifications in food availability and niche vacancy appear to partly explain some changes in food web relationships and invertebrate functions in acidic streams by inferring less pronounced heterotrophy. A recent study by Ledger and Hildrew (2000) demonstrated that the trophic linkage in acid streams between benthic algae and invertebrates was maintained by several nonspecialised species, including nemourid and chironomid larvae. My results agree with those findings, revealing that other species may shift from shredding leaves and eating invertebrates to ingesting biofilm. It is likely that the low number of grazer and collector organisms in acidified streams would favour an increase in the feeding niches of the few remaining tolerant species. Furthermore, the presence of specialised consumers of bryophytes such as *Tipula* (*Savtchenkia*) and *C. maclachlani* in the streams studied confirmed that invertebrate consume bryophytes where they are abundant (Steinman and Boston 1993).

In summary, algae and bryophytes are likely to contribute substantially to the energetics of acid streams, although allochthonous detritus may quantitatively represent the main food resource available for invertebrates. Enhanced availability of primary producers may partly explain the feeding plasticity of animals that elsewhere may be typical shredders. This supports the hypothesis that species function might be context dependent, i.e., subject to the influence of environmental conditions.

Use and abuse of FFGs

In many studies, stream invertebrates have been classified into FFGs based on their mode of foraging and the size

Fig. 3. Generalised food web diagram for acid streams using the trophic species identified by cluster analysis in the present study. Dotted and shaded frames indicate resources and primary consumers, respectively. Bold arrows identify strong links between consumers and resources according to gut content analyses. Linkages between intermediate and secondary consumer taxa and their prey are drawn according to the occurrence of the main prey items found in the digestive tracts, completed by field observation for sucking predators (data not shown).

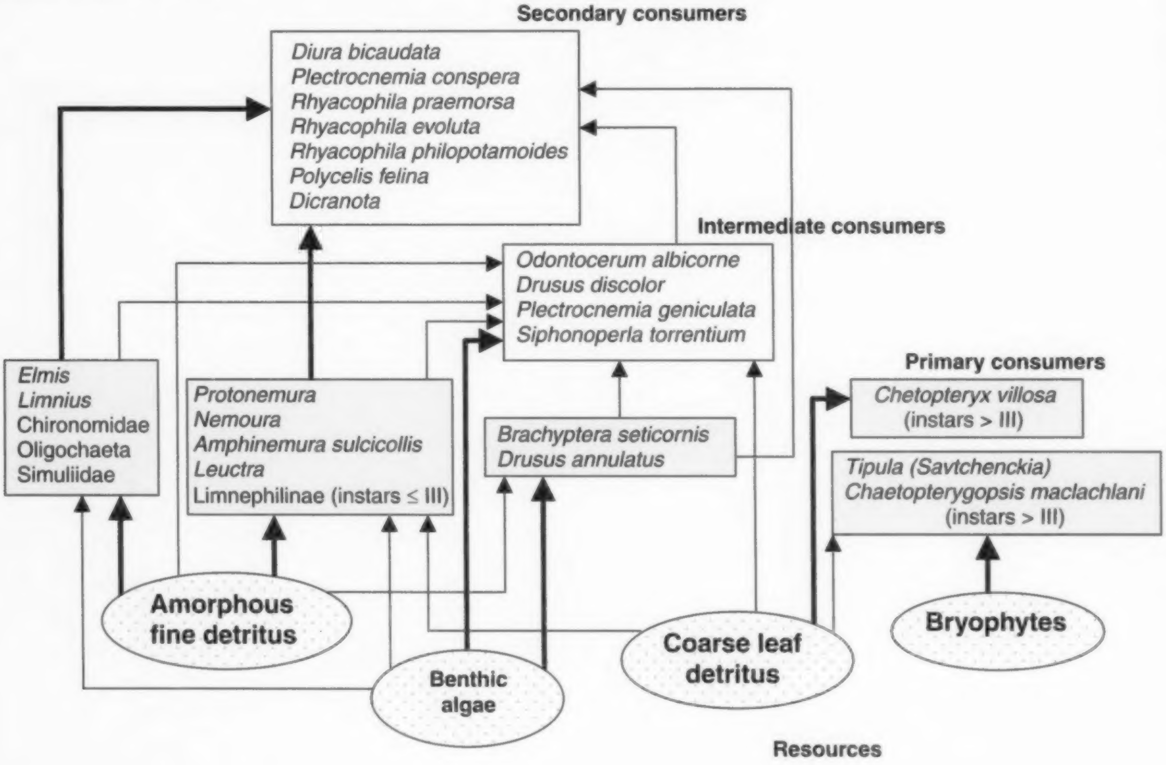

Secondary consumers
Diura bicaudata
Plectrocnemia conspera
Rhyacophila praemorsa
Rhyacophila evoluta
Rhyacophila philopotamoides
Polycelis felina
Dicranota
Intermediate consumers
Odontocerum albicorne
Drusus discolor
Plectrocnemia geniculata
Siphonoperla torrentium
Primary consumers
Chetopteryx villosa (instars > III)
Tipula (Savtchenckia)
Chaetopterygopsis maclachlani (instars > III)
Elmis
Limnius
Chironomidae
Oligochaeta
Simuliidae
Protonemura
Nemoura
Amphinemura sulcicollis
Leuctra
Limnephilinae (instars ≤ III)
Brachyptera seticornis
Drusus annulatus
Amorphous fine detritus
Benthic algae
Coarse leaf detritus
Bryophytes
Resources

Table 5. Comparison of densities and biomasses of shredders in four acid streams (GS = Gentil Sapin, WA = Wassongoutte, BR = Bas-Rupts, RR = Rouge-Rupts; this study) and one circumneutral stream (HR = Hauts-Rupts; Dangles 2000).

Site	Putative shredder density (individuals·m^{-2})	Putative shredder biomass (mg·m^{-2})	"Real" shredder density (individuals·m^{-2})	"Real" shredder biomass (mg·m^{-2})	$-k$ (day^{-1})
Acid streams					
GS	898.2^{a}	129.4^{a}	165.8^{a}	61.5^{a}	0.0006^{a}
WA	1208.0^{b}	260.0^{b}	204.5^{a}	62.5^{a}	0.0008^{a}
BR	806.7^{a}	234.3^{b}	133.9^{a}	85.2^{a}	0.0012^{b}
RR	1214.6^{b}	215.6^{b}	174.1^{a}	73.2^{a}	0.0008^{a}
Circumneutral stream					
HR	990.6^{a}	295.5^{b}	609.6^{b}	210.5^{b}	0.0084^{c}

Note: All densities and biomasses are mean annual values ($n = 7$) adjusted for habitat availability. Values associated with putative shredders are based on functional feeding group (FFG) allocation. Those associated with "real" shredders are based on gut content analyses. Regression analyses of mass loss of beech leaves (December 1997 – August 1998) in the six streams are also presented (data from Dangles and Guérold 2001*a*). $-k$ = estimated daily leaf mass loss in coarse-mesh bags. Within each column, values with different superscript letters are significantly different (ANCOVA for k values, t test for densities and biomasses, $P < 0.05$).

range of particles ingested (Cummins 1973). This concept was developed in response to inadequacies in systematic and trophic analysis: identification to the species level is still difficult and gut content analyses reveal that many invertebrates are polyphagous. Although the appropriateness of FFGs for defining taxa in trophic terms has been controversial ever since the concept was first introduced (see Hawkins and MacMahon 1989), they have been indiscriminately and extensively used by numerous stream ecologists during the last decades to infer and model complex ecosystem processes. The present study shows that FFGs may lack the necessary resolution to understand certain trophic processes of acid streams. This was partly because benthic organisms influence basal resource transfer in species-specific ways. For instance, although closely related, *Tipula* (*Saginata*) and *Tipula* (*Savtchenkia*) performed different functions in the acid streams studied.

Functional plasticity of aquatic invertebrates may also have strong implications for biological conservation of aquatic systems. With the rate of species extinction increasing rapidly, maintaining the integrity of ecosystem function has been thought to be an appropriate way to minimize the consequences of species loss (Walker 1992). A focus on functional groups of organisms, rather than taxonomic species, may therefore appear to be a better strategic alternative in biological conservation. It has also been proposed that within functional groups, some species may be redundant (they perform the same function), consequently requiring less conservation efforts, since other species may replace them (Walker 1992). The present example of the plasticity of species function demonstrates that redundancy might be difficult to quantify in stream ecosystems.

This study provides consistent evidence of the risk of lumping species into convenient groups and reducing taxonomic resolution. Overlooking feeding plasticity (e.g., in filipalpians), allocating precise functions to poorly known species based on the knowledge of closely related species (e.g., *C. maclachlani*) or lumping genera, or even families, containing species with very different autecology (e.g., *Tipula*) are only some of the dangers of using FFGs that must be avoided in the work towards better understanding of ecosystem functioning.

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Appendix A

Table A1. List of the presumed shredder taxa collected in the study streams (GS, Gentil Sapin; BR, Bas-Rupts; WA, Wassongoutte; RR, Rouge-Rupts).

	GS	BR	WA	RR
Plecoptera				
Taeniopterygidae				
Brachyptera seticornis Klapalek		+	+	+
Nemouridae				
Protonemura nimborum Ris (A)		+	+	+
Protonemura meyeri Pictet (A)			+	+
Protonemura intricata (Pictet) Ris (A)		+		
Protonemura lateralis (Pictet) Ris (A)		+		
Protonemura fumosa Ris (A)	+	+		+
Protonemura sp.	+	+	+	+
Amphinemura sulcicollis Stephens	+	+	+	+
Nemoura cinerea Retzius (A)		+		
Nemoura sp.	+	+	+	+
Nemurella picteti Klapalek	+	+	+	+
Leuctridae				
Leuctra prima Kempny (A)	+	+	+	+
Leuctra nigra Olivier (A)	+	+	+	+
Leuctra hippopus Kempny (A)			+	+
Leuctra cingulata Kempny (A)	+	+	+	+
Leuctra inermis Kempny (A)		+	+	+
Leuctra pseudosignifera Aubert (A)		+	+	+
Leuctra sp.	+	+	+	+
Capniidae				
Capnia vidua Klapalek (A)	+	+		+
Trichoptera				
Leptoceridae				
Crunoecia irrorata (Curtis)	+			
Limnephilidae				
Drusus annulatus Stephens	+	+	+	+
Drusus discolor Rambur	+	+	+	
Anomalopterygella chauviniana Stein				+
Acrophylax zerberus Brauer	+			

Table A1 (*concluded*).

	GS	BR	WA	RR
Micropterna lateralis (Stephens)			+	+
Potamophylax cingulatus Stephens		+		
Allogamus uncatus Brauer		+		
Chaetopterygopsis maclachlani Stein	+	+	+	+
Chaetopteryx villosa Fabricius	+	+	+	+
Limnephilinae (instars ≤ III)	+	+	+	+
Sericostomatidae				
Sericostoma personatum Kirby & Spence				+
Diptera				
Tipulidae				
Tipula (*Saginata*) sp.	+	+	+	+
Tipula (*Savtschenkia*) sp.	+	+	+	

Note: (A) following a species name indicates determination based on adult insect.

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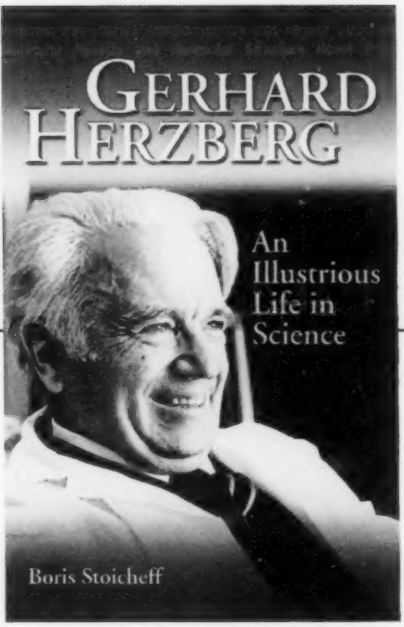

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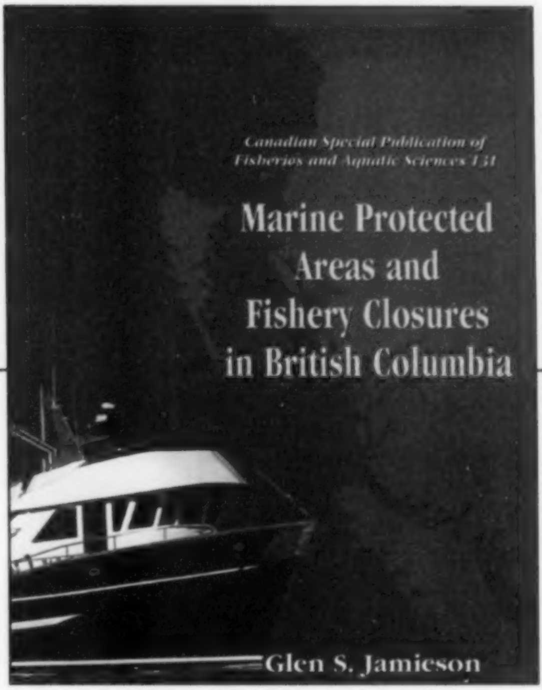

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